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Official Journal of the Brazilian Society of Toxicology

Applied Research in Toxicology

Volume 1 / Suppl. 1 / 2015

9th CTDC XIX CBTox | NATAL BRASIL 2015 | ADVANCING TOXICOLOGY SCIENCE IN DEVELOPING COUNTRIES

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Applied Research in Toxicolgy the official journal of the Brazilian Society of Toxicology, publishes peer-reviewed original scientific research in all fields of toxicology, including but not limited to nanotoxicology, genomics and proteomics, teratogenesis, carcinogenesis, mutagenesis, reproductive and endocrine toxicology, toxicopathology, target organ toxicity, neurobehavioral toxicology, mechanistic studies, biochemical and molecular toxicology, novel biomarkers, risk assessment and environmental health studies. Manuscripts on clinical toxicology and its aspects, toxicodynamics and toxicokinetics are also accepted. In addition to original research articles, concise and current review and mini-review articles are also welcome, as are case report papers.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

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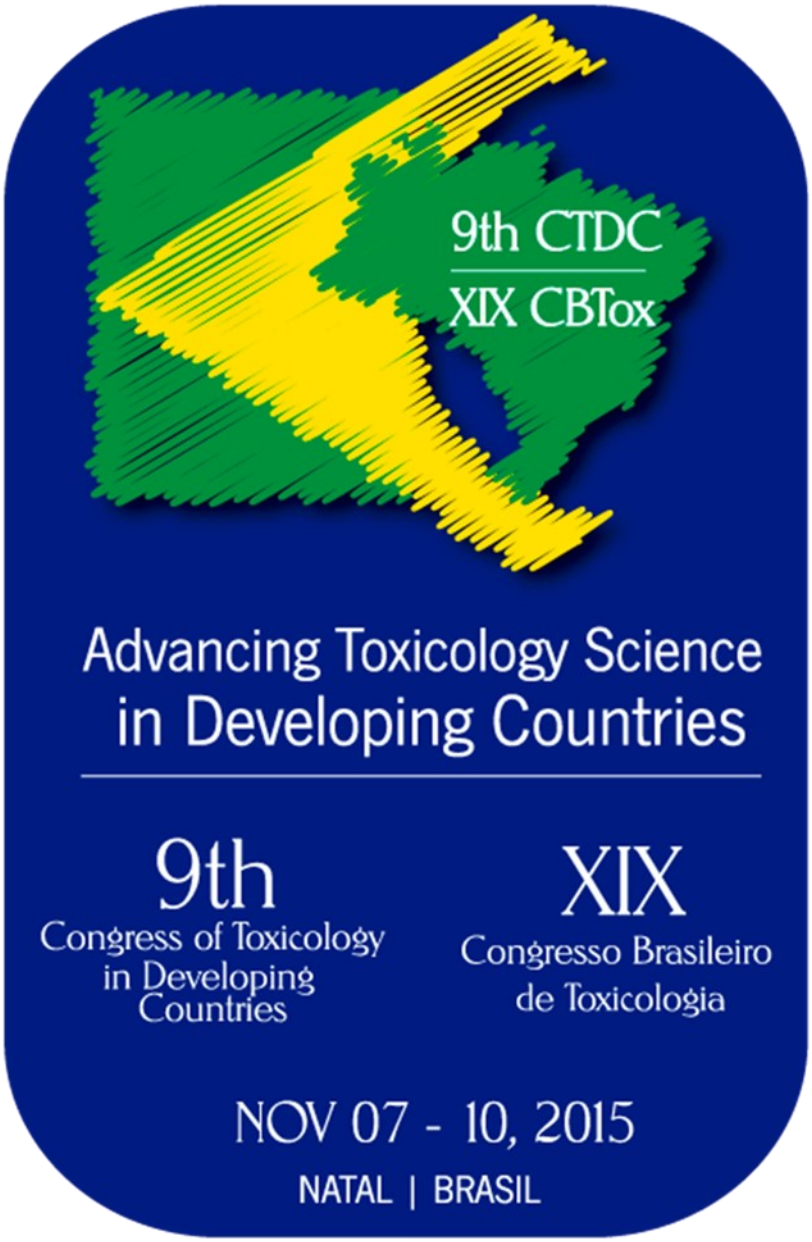
A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015



ABSTRACTS

Promotion

Sociedade Brasileira de Toxicologia

iutox 35th Anniversary
INTERNATIONAL UNION OF TOXICOLOGY

A | R | T
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ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

9th CTDC AND XIX CBTOX PROMOTION / SUPPORT / SPONSORS

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MOLTOX Molecular Toxicology, Inc.

Agilent Technologies

SHIMADZU Excellence in Science

BASF We create chemistry

Waters The Science of What's Possible.

WILEY

Food and Drug Administration — NCTR National Center for Toxicological Research

Nestlé Good Food, Good Life

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TAMARANA Tecnologia & Soluções Ambientais

Bateraxx

Bateria Real

BATS

Júpiter

KF

SUPPORT

ABIHPEC Brazilian Association of the Cosmetic, Toiletry and Fragrance Industry

ApexBrasil

itehpec Institute of Technology and Studies of Personal Hygiene, Perfumery and Cosmetics

ABAV

UFRN

Natal Convention Bureau

RitsQ

EXECUTIVE SECRETARIAT

Objetiva Eventos & Turismo

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

9th CTDC and XIX CBTox WELCOME MESSAGES

Dear Colleagues,

On behalf of IUTOX, I would like to welcome you to the 9th Congress of Toxicology for Developing Countries in the beautiful location of Natal expertly organized by the Brazilian Society of Toxicology (SBTox) under the strong leadership of Daniel Dorta. This congress gives you an opportunity to interact with scientists from other countries in the region or abroad and has attracted prominent scientists to lecture on diverse topics covering many aspects of toxicology.

Formed in 1980 to serve as the scientific voice of toxicology in the world, IUTOX celebrates its 35th anniversary this year as the global umbrella organization for 63 national societies of toxicology representing more than 25,000 members. The IUTOX brand of training and education is distinctive and includes our tri-annual meetings, Congresses of Toxicology for Developing Countries (CTDC) and International Congresses of Toxicology (ICT). The 1st CTDC meeting took place in Argentina in 1987 under the able leadership of Prof. José A. Castro. It has since been organized in India, Egypt, Turkey, China, Croatia, South Africa and Thailand, traveling full circle back to South America today. In addition to the exchange of scientific research and information, an integral part of the CTDC experience includes continuing education courses. This year, we have also collaborated with SBTox to host a course on water safety sponsored by the International Council of Science (ICSU), being held just prior to the Congress.

I hope CTDC9 provides each of you the valuable opportunity to network with your colleagues around the world, and especially to listen to the promise of new and innovative science.

Enjoy the meeting!

Herman Autrup (President, IUTOX)

Dear Colleagues,

As the President of the Brazilian Society of Toxicology (BSOT) I am proud to welcome you to the XIX Brazilian Congress of Toxicology (CBTox) that this year will be held together with the 9th Congress of Toxicology in Developing Countries.

Since our last Brazilian Congress of Toxicology, held in 2013 in Porto Alegre, RS, where I have been selected as the president of the BSOT for a 2-year term, we have being working hard to present you with a program that will give you the best opportunity to discuss the latest trends and scientific breakthroughs in toxicological sciences, at the same time that we also tried to cover all areas of toxicology.

I also need to mention the great opportunity to work together with the International Union of Toxicology - IUTOX. This collaboration was essential to give a wider visibility for our National meeting, which will have about 600 Brazilian and foreign participants, from South, Central and North America, Africa, Asia and Europe; including researchers, professionals from the health area as well as graduate and undergraduate students.

I hope you all have a nice time in Natal, with valuable time to network with colleagues all aorund the world and to enjoy the natural beauties that the city can offer you.

Daniel Junqueira Dorta (BSOT President)

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

PROF. ESTER DE CAMARGO FONSECA MORAES AWARD

This award is given every two years during the Brazilian Toxicology Congress, and is the ultimate recognition of the Brazilian Society of Toxicology for people with a long history of distinguished contributions to the Brazilian toxicology.

We are pleased to announce :

Prof. Dr. Alice Aparecida da Matta Chasin

As the 2015 honoree with the Award

Dr. Alice A. da Matta Chasin has a PhD in Toxicology and Master in Toxicological Analysis by the Faculty of Pharmaceutical Sciences of the University of São Paulo, Brazil (FCF/USP); Full Professor of Toxicology and Coordinator of the Area of Health of the Graduate Programs at Oswaldo Cruz Faculty; Criminal Expert Toxicologist for IML / SP (1976-2004); Professor and Supervisor of the Graduate Program in Toxicology and Toxicological Analysis of FCF / USP (1997-2014). Specialist in Drug abuse with title awarded by UN (United Nations - Narcotics Division). Professor of Forensic Toxicology at the São Paulo Police Academy. Ex- President of the Brazilian Society of Toxicology biennium 2003-2004; Member of AAFS (American Academy of Forensic Sciences). Brazilian Representative at TIAFT (The International Association of Forensic Toxicologists).

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

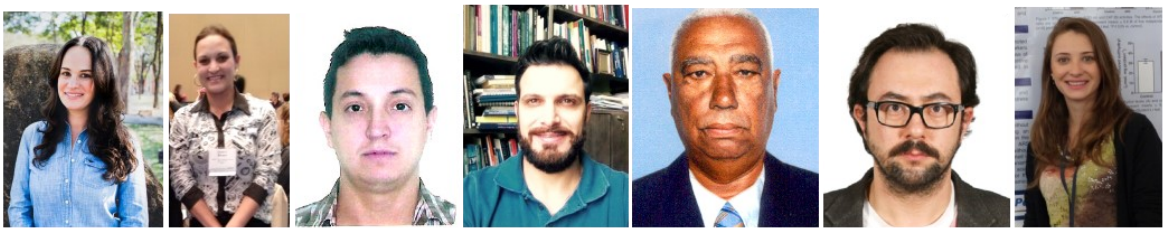
ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

IUTOX TRAVEL AWARD

9th CTDC Travel Awardees

November 7-10, 2015

Natal, Brazil



Adriana Arisetto *Cibele Borges* *Carlos Gonzalez Blanco* *Rafael Buralli* *Angel Casaco* *Erdem Coskun* *Glaucia Dal Santo*

Marlise Domenico *Sarah Eller Franco de Oliveira* *Carlos Falcon* *Elisa Ferraz* *Marcelo Filonzi dos Santos* *Tiago Franco de Oliveira* *Carolina Guzman*

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Adriana Arisetto - Brazil
Carlos Gonzalez Blanco - Brazil
Cibele Borges - Brazil
Rafael Buralli - Brazil
Angel Casaco - Cuba
Erdem Coskun - Turkey
Glaucia Wisoski Dal Santo - Brazil
Marlise Di Domenico - Brazil
Pablo Maldonado Donoso -Ecuador
Pablo Maldonado Donoso -Ecuador
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Adriana I. Haas - Argentina
Zhengtao Liu - China
Mariana N.Mardirosian - Argentina
Gabriela Meireles - Brazil
Ana Flavia Miguel - Brazil
Isabel C. Palacio - Brazil
Lilian Cristina Pereira - Brazil
Gisele A. Rodrigues Oliveira - Brazil
Azman Seeni - Malaysia
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Maria Fernanda Simoniello - Argentina
Songsak Srianujata - Thailand
Unursaikhan Surenjav - Mongolia

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

PRE-CONGRESS PROGRAM

November 7th

Time	Type	Theme	Speaker	Room
9:00 AM – 12:00 PM	CE 1	Toxicologia do Desenvolvimento e da Reprodução (Developing and Reproduction Toxicology)	- Francisco J.R. Paumgartten - Wilma de Grava Kempinas	Bahia Formosa
9:00 AM- 4:00 PM	CE 2	Neurotoxicology	- Michael Aschner - Tomas R. Guilarte - Estefânia G. Moreira - João Batista T. Rocha - Marcelo Farina	Fernando Paiva
9:00 AM- 4:00 PM	CE 3	Fundamentos e aplicações da cromatografia gasosa acoplada à espectrometria de massas (CG-EM) em Toxicologia analítica e Forense (GC-MS in Forensic and Analytical Toxicology)	- Dr. Celso Blatt - Romão Beserra Jr - Elvis Medeiros de Aquino. - Frank T. Peters - Maurício Yonamine - José Xavier - Fabrício Pelição - Luciano Chaves Arantes - Sandra Araruna	Morton
1:00 PM- 4:00 PM	CE 4	Epidemiologia aplicada a Toxicologia: delineamentos (Epidemiolgy for Toxicology)	-Gabriela Arantes Wagner	Bahia Formosa

CONGRESS PROGRAM

November 7th

Time	Type	Theme	Speaker	Room
6:00 PM – 7:00 PM		Opening Ceremony		Lavoisier Maia
7:00 PM - 8:00 PM	**CF 1**	**RELEVANCE OF TOXICOLOGY FOR DEVELOPING COUNTRIES FUTURE**	**- José Alberto Castro**	**Lavoisier Maia**
8:00 PM		WELCOME RECEPTION		HOTEL SEHRS

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 8th

Time	Type	Theme	Speaker	Room
9:00 AM – 11:00 AM	SA 1	**Antimicrobial drugs in food production: residues and resistance concerns**		Fernando Paiva
9:00 AM - 9:20AM	SA1.1	-The safety assessment of antimicrobial use in food producing animals	- João Palermo Neto	
9:20 AM- 9:40AM	SA1.2	-Impact of Antimicrobial Therapy and Antimicrobial Residues on the Intestinal Microbiota	- Carl E. Cerniglia	
9:40 AM- 10:00AM	SA1.3	- The impact of antimicrobial Exposure on Antimicrobial resistance development	- Steven Foley	
10:00 AM- 10:20AM	SA1.4	- Trends in Antimicrobial Resistance among Bacterial Foodborne Pathogens	- Mussaret B. Zaidi	
10:20 AM- 10:40AM	SA1.5	- Conventionally versus organically farmed vegetables: contamination levels of pesticides and heavy metals	- Sameeh A. Mansour	
10:40 AM – 11:00 AM		Discussion		
9:00 AM – 11:00 AM	SA 2	**Analytical Toxicology**		Morton
9:00 AM - 9:30 AM	SA2.1	- Recent applications of LC-MS in Toxicology	- Aldo Polettini	
9:30 AM - 10:00 AM	SA2.2	- New preparation techniques for the analysis of samples of toxicological interest	- Álvaro José dos S. Neto	
10:00 AM – 10:30 AM	SA2.3	-New Psychoactive Substances (NPS): the new challenges to analytical toxicology	- José Luiz da Costa	
10:30 AM – 11:00 AM		Discussion		
9:00 AM – 11:00 AM	SA 3	**Genetic Toxicology Testing, Heritable Mutations, and Carcinogenicity**		Lavoisier Maia
9:00 AM- 9:25 AM	SA3.1	- History of the Genetic Toxicology	Errol Zeiger	
9:25 AM – 9:50 AM	SA3.2	- The recognition of germ cell mutagens and future directions	David de Marini	
9:50 AM – 10:15 AM	SA3.3	- Testing for gene mutations *in vivo*	Bob Young	
10:15 AM – 10:40 AM	SA3.4	- The importance of mutations in carcinogenesis	Luis Felipe Ribeiro Pinto	
10: 40 AM– 11:00 AM		Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 8th

Time	Type	Theme	Speaker	Room
11:00 AM – 12:00 PM	OP 1	**ORAL PRESENTATIONS**		
11:00 AM - 11:20AM	OP1.1	-- Oxidative stress molecular markers evaluation and epidemiologic assessment of subjects environmentally exposed to mercury in the eastern amazon	- Heloisa N.M. Meneses	
11:20 AM- 11:40AM	OP1.2	- The herbicides trifluralin and Tebuthiuron fail to activate The nrf2 antioxidant response	- Mariana F. F. Bernardes	Bahia Formosa
11:40 AM- 12:00 PM	OP1.3	- Oxidative stress and celular alterations in diferent tissues of Litopenaeus vannamei induced by graphene exposure	- Amanda Lucena Fernandes	
11:00 AM – 12:00 PM	OP 2	**ORAL PRESENTATIONS**		
11:00 AM - 11:20AM	OP2.1	- Effect of juices from amazon fruits on the enzymatic activity of human p-glycoprotein in vitro	- Mariana B. A. Figueira	
11:20 AM- 11:40AM	OP2.2	- Cytotoxic and genotoxic evaluation of chlorogenic acid, a dietary bioactive compound, in association with 5-azacytidine in leukemic human cells hl -60	- Lívia C. Hernandes	Fernando Paiva
11:40 AM- 12:00 PM	OP2.3	- Fumonisin b1 and ochratoxin a mycotoxins and biomarkers determination in animal tissues and serum as an assessment of dietary exposure	- Mulunda Mwanza	
11:00 AM – 12:00 PM	OP 3	**ORAL PRESENTATIONS**		
11:00 AM - 11:20AM	OP3.1	-Ecotoxicological risk assessment of the "acid black 210" dye	Otávio P.Rocha	
11:20 AM- 11:40AM	OP3.2	-Investigation of the genotoxic potential of a new brazilian botanical extract – a a case study	Vanessa M. Sá Rocha	Lavoisier Maia
11:40 AM- 12:00 PM	OP3.3	- Health risk assessment of chemicals – A commentary on requirements for the provision of training	Corrado L Galli	
11:00 AM – 12:00 PM	ES 1	*Exhibitor Hosted Session:* **SINC do Brasil** -Soluções Bruker para triagem e identificação automatizada de drogas por Espectrometria de Massas		Morton

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 8th

Time	Type	Theme	Speaker	Room
11:30 AM – 12:00 PM	ES 2	*Exhibitor Hosted Session:* **ANDEF** - Consumer Safety Assessement and Pesticides Residues	- Elizabeth Nascimento	ANDEF BOOTH
12:00 PM – 2:00 PM	LC	**LUNCH**		
12:00 PM – 12:30 PM	ES 3	*Exhibitor Hosted Session:* **ANDEF** - Pesticides Operator Risk Assessement / Avaliação Risco Ocupacional de Agroquímicos /Demo of Personal Protective Equipment	- Karen Cazarin (BASF) - Ana Carolina Aguirre (Syngenta) - João Israel (AzBrasil)	ANDEF BOOTH
12:30 PM – 1:30 PM	ES 4	*Exhibitor Hosted Session:* **ANDEF** - Debate on Regulatory Toxicology: Job Market Perspectives	-Shadia Ihlaseh Catalano (BASF) -Cristiana Leslie Correa (IBTOX) -Cynthia Pestana (TECAM)	Fernando Paiva
12:30 PM – 1:30 PM	ES 5	*Exhibitor Hosted Session*: **Agilent Technologies Brazil** - Method validation for quantitation and confirmation of amphetamines, phentermine, and designer stimulants by LC/MS/MS.	-Celso Blatt (Agilent Technologies)	Morton
2:00 PM – 3:00 PM	PS 1	**POSTER SESSION**	**Areas:** - Genomics, Proteomics and Metabolomics -Experimental Toxicology -Alternative methods to animal experimentation -Food Toxicology	Morton Moriz Pavilion
3:00 PM – 4:00 PM	**CF 2**	**CHEMICALS SAFETY IN LIMBO: HOW LOW SHOULD WE GO?**	**- Linda Birbaum**	**Lavoisier Maia**
4:00 PM – 6:00 PM	SA 4	**Recent advances in reproductive and Developmental Toxicology**		
4:00 PM – 4:20 PM	SA4.1	- In silico models	-Thomas Knudsen	Fernando Paiva
4:20 PM – 4:40 PM	SA4.2	- “Omics”	- Elaine Faustman	
4:40 PM – 5:00 PM	SA4.3	-Epidemiology	- Francisco J.R. Paumgartten	
5:00 PM – 5:20 PM	SA4.4	- Animal experimentation	- Wilma de Grava Kempinas	
5:20 PM – 5:40 PM	SA4.5	- Mechanisms of organophosphate pesticides toxicity in male reproduction	- Maria Betzabet Quintanilla Vega	
5:40 PM – 6: 00 PM		Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 8th

Time	Type	Theme	Speaker	Room
4:00 PM – 6:00 PM	SA 5	**-Regional program development to strengthen and implement virtual data basis of antidotes and intoxication medicaments**		Bahia Formosa
4:00 PM – 4:20 PM	SA5.1	-Accessibility of antidotes in the Ministry of Health of Nicaragua	- Jesus Marin Ruiz - Nicaragua	
4:20 PM – 4:40 PM	SA5.2	- Antidotes and antagonists review in Guatemala Hospitals	- Miriam Carolina Guzmán-Quilo – Guatemala	
4:40 PM – 5:00 PM	SA5.3	- Hydroxocobalamin: national availability for first responders	- Amalia Laborde – Uruguay	
5:00 PM – 5:20 PM	SA5.4	- National and Regional Banks of antidotes	- Adriana Haas – Argentina	
5:20 PM – 5:40 PM	SA5.5	- Antivenoms: importance of a regional cooperation for improving the availability and accessibility	- Hildaura Acosta de Patiño – Panama	
5:40 PM – 6: PM	SA5.6	- Assessment of the availability of antidotes and antagonists in Chilean hospitals	- Juan Carlos Rios Bustamante – Chile	
4:00 PM – 6:00 PM	SA 6	**- New solutions for risk assessment of engineered nanomaterials**		Morton
4:00 PM – 4:30 PM	SA6.1	- Crucial material parameters for nanomaterial toxicity assessment	- Luis Alexandre Muehlmann	
4:30 PM – 5:00 PM	SA6.2	- Safety of nanomaterials today: challenges for workers and consumers	- Mary Gulumian	
5:00 PM – 5:30 PM	SA6.3	- Effects of immobilized surface nanostructures on tissue-material interactions	- Peter Goering	
5:30 PM – 6:00 PM		- Discussion		
4: 00 PM – 6:00 PM	SA 7	**Genotoxicity and cytotoxicity exerted by emerging pollutants in developing countries**		Lavoisier Maia
4:00 PM – 4:40 PM	SA7.1	- Genotoxicity and cytotoxicity of antiviral drugs	- Ofélia A. Olivero	
4:40 PM – 5:05 PM	SA7.2	-Impact on human environmental health from spent coffee grounds discarded in the environment	-Elisa R A Ferraz	
5:05 PM – 5:30 PM	SA7.3	-Monitoring cytotoxic and genotoxic potential in water of Sinos River Basin	-Ana Luiza Ziulkoski	
5:30 PM – 6:00 PM		-Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 9th

Time	Type	Theme	Speaker	Room
9:00 AM – 11:00 AM	SA 8	**Integration of mode-of-action data into the risk assessment of chemicals**		
9:00 AM – 9:25AM	SA8.1	- The mode of action/human relevance framework	- Alan Boobis	
9:25 AM – 9:50 AM	SA8.2	- US Perspective: Application of Mode of Action and Human Relevance Frameworks into Risk Assessment	- Vicki Dellarco	
9:50 AM – 10:15 AM	SA8.3	- Hazard assessment approach in the new brazilian proposal of pesticide regulation	- Andrea Maria Andrade	Lavoisier Maia
10:15 AM – 10:40 AM	SA8.4	- Possible future approaches to human health risk assessment	- Elizabeth S. Nascimento	
10:40 AM – 11:00 AM		- Discussion		
9:00 AM – 11:00 AM	SA 9	**Theories of addiction**		
9:00 AM - 9:30 AM	SA9.1	- Incentive-sensitization theory of addiction	- Cleopatra Planeta	
9:30 AM - 10:00 AM	SA9.2	- New Technologies for examining the role of neuronal ensembles in drug addiction	- Fabio C. Cruz	Fernando Paiva
10:00 AM – 10:30 AM	SA9.3	- Animal models of drug reward, subject effects and relapse	- Rodrigo Molini Leão	
10:30 AM – 11:00 AM		Discussion		
9:00 AM – 11:00 AM	SA 10	**Environmental Risk Assessment**		
9:00 AM- 9:25 AM	SA10.1	- Water and radiation	Viviane Amaral	
9:25 AM – 9:50 AM	SA10.2	- Risk assessment	Fenando Diaz Barriga	
9:50 AM – 10:15 AM	SA10.3	- Old and new contaminants in fresh-water systems. Presence, distribution and transport through the food chain.	Daniel Wunderlin	Morton
10:15 AM – 10:40 AM	SA10.4	- Linking air pollution to epigenetic susceptibility: a road map to precision medicine	Braulio Jimenez-Velez	
10: 40 AM- 12 00 AM		-Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 9th

Time	Type	Theme	Speaker	Room
11:00 AM – 12:00 PM	OP 4	**ORAL PRESENTATIONS**		Bahia Formosa
11:00 AM - 11:20AM	OP4.1	-- Prenatal betamethasone exposure alters initial sexual development and reproductive parameters in adult female rats	- Tainá Louise Pacheco	
11:20 AM- 11:40AM	OP4.2	- Recreational use of marijuana during pregnancy is associated with adverse gestational outcomes and impaired reflex and decreased muscular strenght in neonates	- Marlise di Domenico	
11:40 AM- 12:00 PM	OP4.3	- Indentification of specific protein in prostate carcinogenesis induced by heterocyclic amine-2-amino-1-methyl-6-phenylimidazo [4-5] pyridine (PHIP) using in vitro models	- Azman Seeni	
11:00 AM – 12:00 PM	OP 5	**ORAL PRESENTATIONS**		Fernando Paiva
11:00 AM - 11:20AM	OP5.1	- Skin permeability and cytotoxicity of topotecan-loaded lipid nanoparticles	- Gisele A. R. Oliveira	
11:20 AM- 11:40AM	OP5.2	- Development of an in vitro epidermal model to evaluate skin sensitization	- Tatiana N. Pedrosa	
11:40 AM- 12:00 PM	OP5.3	- Reconstructed Human Epidermis (RHE): From skin irritation to skin sensitization	- Rodrigo de Vecchi	
11:00 AM – 12:00 PM	OP 6	**ORAL PRESENTATIONS**		Lavoisier Maia
11:00 AM - 11:20AM	OP6.1	- Inhalation of fine particles from mexico city increases the exacerbations in a guinea pig asthma model	Carlos Falcon-Rodriguez	
11:20 AM- 11:40AM	OP6.2	- Research progress of water quality criteria in china	Zhengtao Liu	
11:40 AM- 12:00 PM	OP6.3	- Screening of native fishes for deriving aquatic life criteria	Zhenguang Yan	
11:00 AM – 12:00 PM	ES 6	*Exhibitor Hosted Session:* **SINC do Brasil** -GC-MS/MS aplicado a análises forenses: Apresentando Smart Forensic Database		Morton
11:30 AM – 12:00 PM	ES 7	*Exhibitor Hosted Session:* **ANDEF** - Proposal of Toxicological Assessment for Regulatory Decision Making Process	- Ligia Amaral (IBTOX) - Simone Valente (Bayer)	ANDEF BOOTH

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 9th

Time	Type	Theme	Speaker	Room
12:00 PM – 2:00 PM	LC	**LUNCH**		
12:00 PM – 12:30 PM	ES 8	*Exhibitor Hosted Session:* **ANDEF** - Launch of Industrial Technical Information - Challenges to set harmonized maximum residues levels (MRLs) to facilitate world trade - Croplife America (August 2014)	- Wanda Gimenez, BASF	ANDEF BOOTH
12:30 PM – 1:30 PM	ES 9	*Exhibitor Hosted Session*: **NC3Rs** - Global harmonization of regulatory acceptance and approaches to reduce animal use	-Claire Terry (Dow AgroScience) -Natalie Burden (NC3Rs) -Fiona Sewell (NC3Rs)	Fernando Paiva
12:30 PM – 1:30 PM	ES 10	*Exhibitor Hosted Session:* **Shimadzu** - Development of ultra-fast methods for toxicological and forensic applications: the evolution of LC-MS/MS	- Daniel Saidemberg, (Product Specialist - Shimadzu do Brasil)	Morton
2:00 PM – 3:00 PM	PS 2	**POSTER SESSION**	**Areas:** - *Environmental Toxicology and Ecotoxicology* - *Mutagenesis and Carcinogenesis* - *Veterinary Toxicology* - *Immunotoxicology* - *Forensic Toxicology*	Morton Moriz Pavilion
3:00 PM – 4:00 PM	**CF 3**	**UNIVERSAL TOXICOLOGY BY MASS SPECTROMETRY**	**- Marcos Nogueira Eberlin**	**Lavoisier Maia**
4:00 PM – 6:00 PM	SA 11	**Anvisa's perspectives on Brazilian toxicological re-evaluation of pesticides**		Bahia Formosa
4:00 PM – 4:20 PM	SA11.1	- Understanding Brazilian regulation on re-evaluation of pesticides	- Caio Augusto de Almeida	
4:20 PM – 4:40 PM	SA11.2	- How to deal with conflicting results on a regulatory perspective?	- Juliana Machado Braz e Camila Braz Moreira	
4:40 PM – 5:00 PM	SA11.3	The challenges of establishing criteria for toxicological analysis: Mutagenesis	- Alexandre Augusto Sasaki	
5:00 PM – 5:20 PM	SA11.4	- Comparative analysis of pesticides´ reevaluation processes among some regulatory agencies	- Rodrigo Gregório Botelho	
5:20 PM – 5:40 PM	SA11.5	- How to prioritize pesticides´ reevaluations in Brazil?	- Camila Queiroz Moreira	
5:40 PM – 6: 00 PM		Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 9th

Time	Type	Theme	Speaker	Room
4:00 PM – 6:00 PM	SA 12	**- Maternal-fetal Toxicity**		
4:00 PM – 4:30 PM	SA12.1	- Fetuses as a high-risk group to methyl mercury exposure	- Mineshi Sakamoto	
4:30 PM – 5:00 PM	SA12.2	- Recent advances in the study of the effects of traffic derived air pollution on health: mother and child as the most vulnerable groups	- Mariana Matera Veras	Fernando Paiva
5:00 PM – 5:30 PM	SA12.3	- Investigating the stereoselective clinical toxicokinetics in pregnancy using in vivo and ex vivo placental transfer model	- Leonardo Santos Ribeiro Pinto	
5:30 PM – 6:00 PM		- Discussion		
4:00 PM – 6:00 PM	SA 13	**- Should Azo dyes be considered as water emerging contaminants?**		
4:00 PM – 4:15 PM	SA13.1	- An overview on the azo dyes toxicity	- Danielle P. Oliveira	
4:15 PM – 4:45 PM	SA13.2	- Artificial skin as a model for risk assessment of hair dyes	- Silvya Stuchi Maria-Engler	Morton
4:45 PM – 5:15 PM	SA13.3	- Toxicological significance of azo dyes metabolism by human intestinal microbiota	- Carl E. Cerniglia	
5:15 PM – 5:45 PM	SA13.4	Ecotoxic effects of azo dyes	Gisela A. Umbuzeiro	
5:45 PM – 6:00 PM		- Discussion		
4: 00 PM – 6:00 PM	SA 14	**Drugs of abuse: mechanisms underlying addicition**		
4:00 PM – 4:25 PM	SA14.1	- Endocannabinoid system: implications in drugs addiction	- Marco Pistis	
4:25 PM – 4:50 PM	SA14.2	-Amphetamine-induced behavioral sensitization and the dopaminergic system	- José Antônio Fuentealba	Lavoisier Maia
4:50 PM – 5:15 PM	SA14.3	- Environmental tobacco smoke: predisposition to addiction and endocannabinoid system	- Larissa Helena Lobo Torres	
5:15 PM – 5:40 PM	SA14.4	- Anhydroecgonine methyl ester, a crack cocaine pyrolysis product, contributes to cocaine behavioral sensitization	- Raphael Caio T. Garcia	
5:40 PM – 6:00 PM		- Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 10th

Time	Type	Theme	Speaker	Room
9:00 AM – 11:00 AM	SA 15	**Mitochondrial Toxicity**		Fernando Paiva
9:00 AM – 9:30 AM	SA15.1	- Nanoparticles: is toxicity a concern?	- Carlos Palmeira	
9:30 AM – 10:00 AM	SA15.2	- Impact of highly toxic chemicals on the respiratory system and the skin: pathophysiology and new therapeutic approaches	- Horst Thiermann	
10:00 AM – 10:30 AM	SA15.3	- Polybrominated Flame retardants (PBDEs) effects on mitochondria	- Daniel Junqueira Dorta	
10:30 AM – 11:00 AM		Discussion		
9:00 AM – 11:00 AM	SA 16	**Alternative methods for skin sensitization**		Morton
9:00 AM - 9:30 AM	SA16.1	- Understanding potency of chemical allergens: contribution of keratinocytes	- Emanuela Corsini	
9:30 AM - 10:00 AM	SA16.2	- Understanding the mechanistic differences and similarities between sensitization and irritation with the aid of tissue engineered skin models	- Sue Gibbs	
10:00 AM – 10:30 AM	SA16.3	- Data integration of non-animal tests to assess chemical Skin Sensitization potency	- Vanessa de Sá Rocha	
10:30 AM – 11:00 AM		Discussion		
9:00 AM – 11:00 AM	SA 17	**Metal Toxicity**		Lavoisier Maia
9:00 AM- 9:25 AM	SA17.1	- Contribution of the Keap1/ Nrf2 pathway and CBS/CSE to produce reactive sulfur species to protection against methylmercury	- Yoshito Kumagai	
9:25 AM – 9:50 AM	SA17.2	- Cadmium toxicity, its mechanistic, clinical and regulation aspects	- Fujio Kayama	
9:50 AM – 10:15 AM	SA17.3	- Human health risk assessment: Study of a population chronically exposed to arsenic through drinking water from Argentina	- Julio Navoni	
10:15 AM – 10:40 AM	SA17.4	- Metal contaminants in drugs, ICH point of view.	- Akihiko Hirose	
10:40 AM – 11:00 AM		Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 10th

Time	Type	Theme	Speaker	Room
11:00 AM – 12:00 PM	OP 7	**ORAL PRESENTATIONS**		
11:00 AM - 11:20AM	OP7.1	- Structural characterization and biological activities of bordonein-l, an l -amino acid oxidase isolated from Crotalus durissus terrificus snake venom	- Gisele Adriano Wiezel	
11:20 AM- 11:40AM	OP7.2	- New insights of interaction (inhibition, reactivation and aging) of organophosphorus compounds with human acetyl cholinesterase using mass spectrometry and QMMM approaches	- Iris Mangas Nadal	Fernando Paiva
11:40 AM- 12:00 PM	OP7.3	- HER-1 cancer vaccine: Immunotoxicological studies from non-clinical evaluation in non-human primates to clinical evaluation in prostate castration-resistant carcinoma patients.	- Angel Casacó	
11:00 AM – 12:00 PM	OP 8	**ORAL PRESENTATIONS**		
11:00 AM - 11:20AM	OP8.1	- Optimization of a multi-mycotoxin method using LC-MS/MS	- Patrícia Diniz Andrade	
11:20 AM- 11:40AM	OP8.2	- Vitreous humour: real-time analysis of opiates using exactive FTMS	- Júlio César Santos Júnior	Morton
11:40 AM- 12:00 PM	OP8.3	- Measurement of DNA repair proteins in relation to disease biomarkers and drug development	- Erdem Coskun	
11:00 AM – 12:00 PM	OP 9	**ORAL PRESENTATIONS**		
11:00 AM - 11:20AM	OP9.1	- Environmental and biological monitoring of occupational formaldehyde exposure resulting from the use of products for hair straightening	- Giovana Piva Peteffi	
11:20 AM- 11:40AM	OP9.2	- Metabolic warning: disruption of energetic metabolites precedes cell transformation by B[a]P exposure	- Tiago Franco de Oliveira	Lavoisier Maia
11:40 AM- 12:00 PM	OP9.3	- Sodium butyrate reduces nuclear p53 and CRM1 exportation in liver cancer cells	- Juliana Festa Ortega	

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 10th

Time	Type	Theme	Speaker	Room
11:00 AM – 12:00 PM	L 1	**Glyphosate is not an endocrine disruptor: Regulatory Safety Studies & Tier 1 Endocrine disruptor Screening Program (EDSP) Assays Provide a weight of evidence**	- Steven L Levine	Bahia Formosa
11:30 AM – 12:00 PM	ES 11	*Exhibitor Hosted Session*: **ANDEF** - Toxicology in the development of new molecules	- Bennard Ravenzwaay (BASF) - Priscila Fagundes (Syngenta)	ANDEF BOOTH
12:00 PM – 2:00 PM	**LC**	**LUNCH**		
12:00 PM – 12:30 PM	ES 12	*Exhibitor Hosted Session*: **ANDEF** - ILSI Brasil- Food Consumption in Brazil (POF 2008/09 IBGE) – Pesticides residues, exposure assessment	- Cristiana Leslie Correa (PLANITOX) - Laura Valerio (Syngenta)	ANDEF BOOTH
12:30 PM – 1:30 PM	ES 13	*Exhibitor Hosted Session:* **Waters** - Chromatography and Mass Spectrometry Analytical Solutions for Toxicology	- Michael Murgu (Waters)	Lavoisier Maia
12:30 PM – 1:30 PM	GC	**GLOBAL COMMUNICATIONS AND COLLABORATION IN TOXICOLOGY** Hosted by IUTOX and HOT (Hispanic Organization of Toxicologists, a Special Interest Group of the Society of Toxicology) All are invited		Morton
2:00 PM – 3:00 PM	PS 3	**POSTER SESSION**	**Areas:** - Clinical Toxicology - Analytical Toxicology - Medicines and Cosmetics Toxicology - Occupational Toxicology - Social Toxicology - Toxinology - Nanotoxicology	Morton Moriz Pavilion
3:00 PM – 4:00 PM	**CF 4**	**HUMAN-ON-A-CHIP: ALTERNATIVE METHODS**	**- Daniel Levner**	**Lavoisier Maia**

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 10th

Time	Type	Theme	Speaker	Room
4:00 PM – 6:00 PM	SA 18	**Clinical Toxicology**		
4:00 PM – 4:25 PM	SA18.1	- Imaging Diagnostic in Clinical Toxicology	Eduardo Mello De Capitani	
4:25 PM – 4:50 PM	SA18.2	- Evidence-based Toxicology: Where are we?	- Taís Freire Galvão	Bahia Formosa
4:50 PM – 5:15 PM	SA18.3	Antidotes Availability in Brazil: An overview	- Fábio Bucaretchi	
5:15 PM – 5:40 PM	SA18.4	- Oximes and their use in organophosphorous acute intoxications	- Horst Thiermann	
5:40 PM – 6: 00 PM	SA18.5	Discussion		
4:00 PM – 6:00 PM	SA 19	**Toxicogenomics**		
4:00 PM – 4:35 PM	SA19.1	- Percellome Toxicogenomics Project	- Jun Kanno	
4:35 PM – 5:10 PM	SA19.2	- A 21st Century Roadmap for Human Health Risk Assessment	- Herman Autrup	Fernando Paiva
5:10 PM – 5:45 PM	SA19.3	- Tox21 Phase III – Improving on Biological Coverage and Human Relevance in High-Throughput and Toxicogenomic Screening Approaches	- Richard Paules	
5:45 PM – 6:00 PM		- Discussion		
4:00 PM – 6:00 PM	SA 20	**- Alternative methods to animal experimentation – Considerations for regulatory issues in Developing and Developed countries**		
4:00 PM – 4:15 PM	SA20.1	- Brazilian Regulatory Acceptance of Alternative Methods to animal testing	- Caio Rodrigo Teixeira (ANVISA)	
4:15 PM – 4:45 PM	SA20.2	In Silico Methods for Prediction of ADMETox Properties of Drugs and Cosmetics	- Eduardo Pagani (RENAMA)	Morton
4:45 PM – 5:15 PM	SA20.3	- Green toxicology and green chemistry: hand in glove	- Bennard Ravenzwaay	
5:45 PM – 6:00 PM		- Discussion		

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

November 10th

Time	Type	Theme	Speaker	Room
4: 00 PM – 6:00 PM	SA 21	**Forensic Toxicology**		
4:00 PM – 4:35PM	SA21.1	-Emerging Drugs of Abuse	- Frank Peters	
4:35 PM – 5:10 PM	SA21.2	- Studies on drug metabolism by fungi colonizing decomposing human cadavers	- Jorge A. Martinez-Ramirez	Lavoisier Maia
5:10 PM – 5:45 PM	SA21.3	-Analysis of postmortem samples and its interpretation		
5:45 PM – 6:00 PM		- Discussion	- Bruno Spinosa de Martinis	
6:00 PM – 7:00 PM		**Closing Ceremony** **Best Posters Award Ceremony**		Lavoisier Maia

Legends

AS	Brazilian Society of Toxicology General Assembly
CE	Continuing Education Courses
CF	Conference
ES	Exhibitor sponsored session
GC	Global Communication and Collaboration in Toxicology meeting
L	Lecture
LC	Lunch
OP	Oral Presentation
PS	Poster session
SA	Symposium

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

GENOMICS, PROTEOMICS AND METABOLOMICS

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OM 01- ABC TRANSPORTERS DIVERSITY AND ADAPTATIONS IN LORICARIIDAE FAMILY

MOREIRA, D.A.[1]; MAGALHÃES, M.G.P.[1]; ANDRADE, P.C.C.[1]; BUCKUP, P.A.[2]; FURTADO, C.[3]; VAL, A.L.[4]; HAHN, M.E.[5]; STEGEMAN, J.J.[5]; PARENTE,T.E.[1]

[1]- Laboratório de Toxicologia Ambiental, Escola Nacional de Saúde Pública (ENSP), Fundação Oswaldo Cruz (FIOCRUZ); [2]- Laboratório de Biodiversidade Molecular, Setor de Ictiologia, Museu Nacional, Universidade Federal do Rio de Janeiro (MN UFRJ); [3]- Divisão de Genética, Instituto Nacional do Cancer (INCA); [4]- Laboratório de Ecofisiologia e Evolução Molecular, Instituto Nacional de Pesquisas da Amazônia (INPA); [5]- Woods Hole Oceanographic Institution (WHOI).

Introduction: ATP Binding Cassette (ABC) transporters form a monophyletic superfamily of genes, classified into eight subfamilies, which play crucial rules in the cellular excretion of toxins. Loricariidae is the fifth most species-rich vertebrate family and the most diverse among Siluriformes. Despite their extreme diversity, there is no information about ABC in loricariids. Objective: This study aims to identify the gene diversity of ABC transporters using transcriptomic data in the Loricariidae fish. Material and Methods: Three species of Loricariidae fish (*Pterygoplichthys anisitsi, Ancistrus sp1, Ancistrus sp2*) and 1 of Callichthyidae (*Corydoras nattereri*) were sampled in Rio de Janeiro and Amazon States in Brazil. Total RNA was extracted from the liver, following Illumina HiSeq2500 sequencing. The transcriptome was assembled using Trinity. ABC transporters sequences were identified using similarity search (BLAST) with a database of ABC homologous proteins from *Danio rerio* and other more related fish species. The ABC sequences were retrieved from the transcriptome, and edited with Seaview, aligned with Muscle and phylogenetic trees were built with maximum likelihood. Results and Discussion: From more than 50 thousands transcripts in each transcriptome, we found 89 to 106 sequences that had a BLAST top hit with an ABC transporter, covering seven of the eight described subfamilies. The species with more sequences coding a complete CDS, including the 5' and 3' UTR, was *P.anisitsi* with 20 contigs. *C.nattereri* was the species with less diversity, with only 8 sequences with complete CDS. Our phylogenetics results show that ABCE subfamily cluster together with ABCD, ABCB and ABCC when the Nucleotide Binding Domain region is considered, while it clusters with ABCF, but both are inside the clade of ABCA and ABCG, when the complete CDS is aligned with MUSCLE. Conclusion: So far, we have identified the diversity of ABC transporters among three different genera of Siluriformes, mainly Loricariids. We have sequenced the liver transcriptome of other 31 species of Loricariidae. We will be able to identify the ABC diversity in the other species, study the selective pressures guiding its evolution, and the responses of ABC transporters of selected loricariids species to toxins.

Acknowldgements: Financial support from USAID (PGA-2000003446).

OM 02- BIODIVERSITY OF SULFOTRANSFERASE (SULT) FAMILY IN ANCISTRUS SP., CORYDORAS SP. AND *PTERYGOPLICHTHYS ANISITSI*

ANDRADE, P.C.C.[1]; MOREIRA, D.A.[1]; MAGALHÃES, M.G.P.[1]; BUCKUP, P.A.[2]; FURTADO, C.[3]; VAL, A.L.[4]; STEGEMAN, J.J.[5]; HAHN, M.E.[5]; PARENTE,T.E.[1]

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Introduction: The neotropical catfish family Loricariidae (Siluriformes) is the fifth most speciose among all vertebrates. Some Loricariidae fish are known to be highly resistant to organic toxins and to have cytochromes P450 with altered substrate specificities. Loricariidae sulfotransferases has not been investigated. Sulfotransferases enzymes catalyze the transfer of a sulfate group to a hydroxyl or amino group on the substrate molecule. Cytosolic sulfotransferase (SULT) are involved on the metabolism of endogenous compounds and on the detoxification of multiple xenobiotics.
Objective: This work aim to investigate the biodiversity of cytosolic sulfotransferases (SULT) transcripts in Loricariidae fish family, and to evaluate their regulation by xenobiotics.
Material and Methods: Thirty-one species of Loricariidae fish and four of Callichthyidae was sampled in Rio de Janeiro and Amazon States in Brazil. The liver was excised and preserved in RNA Later until total RNA extraction, which was used for cDNA libraries preparation for Illumina sequencing. Quality of RNA extractions and libraries were accessed by Bioanalyzer. Libraries were barcoded and sequenced on eight lanes of a HiSeq2500. The liver transcriptome was assembled with Trinity. EH transcripts were retrieved using BLAST with a local database of EH genes from *Danio rerio* and other closer related fish species. The sequences were edited with Seaview, aligned with Muscle and phylogenetic trees built using PhyML.
Results and Discussion: Transcriptomes of three fish species have been analyzed. In total, 15 transcripts of *Ancistrus* sp., 26 of *Corydoras* sp. and 67 for *Pterygoplichthys anisitsi* were identified to code for SULT enzymes. Of these sequences, 8 from *Ancistrus* sp., 12 from *Corydoras* sp. and 52 from *P. anisitsi* coded for more than 75% of the complete coding sequence (CDS) of their zebrafish homolog. Currently, phylogenetic relations among these sequences are being evaluated, as well as evidences of episodic diversifying selection.
This work is supported by the USAID grant number PGA-2000003446.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OM 03- MEASUREMENT OF DNA REPAIR PROTEINS IN RELATION TO DISEASE BIOMARKERS AND DRUG DEVELOPMENT

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Introduction: In aerobic organisms, intracellular metabolism and exogenous sources such as ionizing radiation and carcinogenic compounds generate reactive species including free radicals derived from either oxygen or nitrogen. Oxidative stress thereby generated may lead to increased genetic instability, proliferation, cell death, apoptosis and onset of inflammation; a hallmark of cancer. Oxidatively induced DNA damage is repaired in vivo by various mechanisms involving numerous DNA repair proteins. If not repaired, this may lead to mutagenesis, which is a fundamental part of the molecular basis of all cancers. In addition, use of repair protein inhibitors in chemotherapy as the novel treatment strategy increases the importance of our knowledge for expressions of those proteins in human tissues. **Objective:** To develop a robust quantitative method for positive identification and accurate quantification of repair proteins in human tissues which have important predictive and prognostic value for cancer development and therapy. **Materials and Methods:** Liquid chromatography-tandem mass spectrometry (LC-MS/MS) with isotope-dilution technique is used to identify and measure the amounts of the proteins. The use of ^{15}N-labelled proteins as an internal standard is critical for accurate measurements of their levels therefore we overexpressed and purified His-tagged and ^{15}N-proteins from *E.coli*. Then, we hydrolyzed both labelled and unlabeled proteins with trypsin and used LC-MS/MS to separate and identify resulting tryptic peptides by their full-scan and product ion mass spectra. Finally, selected-reaction monitoring of the tryptic peptides were determined for positive quantification. **Results and Discussion:** Here, we developed a methodology to positively identify and accurately measure some of the important repair proteins in human tissues using LC-MS/MS with isotope-dilution. Followed by the development of this novel method, we also measured levels of hAPE1 and hMTH1 in human normal and malignant breast tissues, and in four human cultured cell lines. We recorded extremely higher levels of hAPE1 and hMTH1 in MCF7 than MCF10A, cancer and normal cell lines of the same origin, respectively. Supporting these results, highly significant differences between levels of hAPE1 and hMTH1 in normal and malignant tissues were observed. **Conclusions:** The novel approach described herein is expected to be applicable to the measurement of DNA repair proteins' expression levels in cancerous vs. normal tissues in patients. Our ultimate goal for future is to evolve this novel approach as simple as a regular screening test, which will give us the expression levels of various DNA repair proteins in *one pot*, helping clinicians to develop treatment strategies and guide therapies instantly.

OM 04- CYP3A4, CYP3A5 AND UGT2B7 POLYMORPHISMS AND EVALUATION OF REJECTION RISK IN KIDNEY TRANSPLANT PATIENTS

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Introduction: Single nucleotide polymorphisms that codify altered enzymes of biometabolism cause a differential response in patients treated with immunosuppressive drugs and can trigger a rejection process in kidney transplant patients. **Objective: This study evaluated the association between** *CYP3A4, CYP3A5 and UGT2B7* polymorphisms and acute kidney graft rejection. **Material and Methods:** This study was approved by Human Research Ethics Committee of the State University of Londrina, Brazil. In this hospital-based study 247 kidney transplant patients were evaluated, and 86 were diagnosed with acute kidney allograft rejection. The DNA was extracted from peripheral blood and the genotyping of polymorphism of genes *CYP3A4* (rs3559367), *CYP3A5* (rs776746) and *UGT2B7* (rs7662029) was performed by real time PCR using TaqMan probes. The association between rejection episode and the polymorphic variants was assessed by calculating the odds ratio (OR) with confidence interval (IC) of 95% using SPSS® 20 Statistics software (IBM; Armonk, NY, USA). **Results and Discussion:** The average age of the patients was 49 years (ranging between 15 to 76 years). 59% of these patients were males and 35% developed rejection episodes. In this study the genotypes A/A (OR=0.35 (0.14-0.90) p=0.029), A/G (OR=0.53 (0.30-0.92) p=0.025 and A/A+A/G (OR=0.49 (0.28-0.84) p=0.01) of *UGT2B7* gene were associated with a decreasing in the risk of rejection episodes. The product of this gene converts the active metabolite of mycophenolate mofetil (MMF), the mycophenolic acid, to its inactive form mycophenolic acyl-glucuronide. However, the function of this polymorphism remains unknown. The MMF was used by 58% of the patients of this study. The genotype CT and TT of *CYP3A4* gene has been linked to reduced expression and activity of this protein in hepatic cells, while the genotype GG of *CYP3A5* gene promotes a defect in RNA splicing that lead to the lack of expression of the enzyme. For this reason, the carriers of both polymorphism are poor metabolizers and consequently, require lower doses of tacrolimus. In this study, no significant association was found between the frequency of the polymorphic alleles of *CYP3A4* and *CYP3A5* genes and rejection episodes. **Conclusions:** The results indicate that the *CYP3A4* (rs3559367) and *CYP3A5* (rs776746) polymorphisms were not associated with rejection episodes but the polymorphism rs7662029 of the *UGT2B7* gene may protect the kidney transplant patients against the development of rejection episodes.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OM 05- A sublethal concentration of the cyanotoxin Cylindrospermopsin causes primarily structural and metabolic protein upregulation on HepG2 cells

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[2] IRIC (Institute for Research in Immunology and Cancer), Université de Montreal, Canada.

Introduction: The production of cyanobacterial toxins in Brazilian continental water reservoirs is widely known and a current public health issue. [1, 2] Cylindrospermopsin (CYN) is a hepatotoxin who's excretion was initially reported by *Cylindrospermopsis spp.*, but later found to de produced by many other cyanobacterial genera. [1] Several toxicity mechanisms have been described for CYN on mammalian cell cultures. Scientific evidence shows that the earliest toxicity mechanism during cell intoxication by CYN may be protein synthesis inhibition, although this mechanism is not year clear. Objective: To quantify by nanoscale LC-MS^2 the effects of a sublethal dosis of CYN on the upregulation and downregulation of proteins from the overall HepG2 proteome. Materiais and Methods: HepG2 cells, SILAC (stable isotope labeling of amino acids in cell culture), SCX fractionation, Nano LC coupled to an Orbitrap Q-Exactive Plus (Thermo® Fisher Scientific). Results and discussion: Previously results from our laboratory using flow cytometry showed that the treatment of HepG2 cells with 1 µM of CYN during 24 hours, did not produce apoptotic nor necrotic effects. Using this CYN concentration HepG2 cells were treated with this toxin in 12 time intervals from zero to 12 hours. Control HepG2 cells were grown on regular DMEM medium (light). Treated cells were previously grown on two different SILAC medium conditions: medium (Arg $^{13}C_6$ and Lys 4,4,5,5-D_4) and heavy (Arg $^{13}C_6$ $^{15}N_4$ and Lys $^{13}C_6$ $^{15}N_2$). Light, medium and heavy cells were collected after each time point treatment. Cells were then lyzed, the total protein was measured and then every sample was digested with trypsin. Every lysate was then fractionated 5 times using SCX spin tips, and all the fractions were injected on a nano LC-MS^2 equipped with a C18 capillary column (15cm x 150um). The chromatographic elute was electro sprayed directly into the MS^2 analyzer. From a total of 2161 quantifiable proteins, 104 proteins were significantly down-regulated and 189 proteins were significantly up-regulated for the whole experiment. We categorized the significantly abundant proteins in 6 major categories based on the protein molecular function, based on the GOMF (gene ontology molecular function), KEGG (Kyoto encyclopedia of genes and genomes) and Uniprot function details. The dataset was also analyzed using the Ingenuity® Pathway Analysis Software. Conclusions: On this shotgun proteomics experiment, proteins related to anatomical structure and general metabolism (including biosynthetic or general catalytic functions) of the cell corresponded to the higher percentages of upregulated and downregulated proteins. Proteins related to metabolic processes were the most significantly downregulated of all groups. This information gives us a first look of what is going on in the proteome of the HepG2 after sublethal CYN estimulaton. Proteins involved in acute phase response signaling and highly energetic metabolic pathways were significantly upregulated.

References:
[1] H. Hudnell et al., Springer Science. (2008).
[2] W. Carmichael W. et al., Toxicon. 6 (2006).

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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OM 06- IDENTIFICATION OF METAL PROTEINS ASSOCIATED WITH MERCURY IN BREAST MILK SAMPLES

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Introduction: The interest in understanding the biogeochemical cycle of mercury in the Amazon is due to the high toxicity of its organic form, methylmercury. Metallomics presents itself as an innovative proposal for the development of mercury toxicity biomarkers associated with metalloproteins. **Objective:** The objective was the protein fractionation of milk samples from lactating mothers residing it the Madeira River (RO) and Goiânia (GO) and the mapping of Hg in protein bands. **Materials and Methods:** This study included 10 samples of human milk from lactating mothers from communities of the Madeira River, Rondônia, Brazil (n = 7), and the municipality of Goiânia - GO, Brazil (n = 3) as the Control Group. The lipid fraction was separated from protein by centrifugation at 14,000 rpm / 60 min. The fractionation of proteins was carried out by SDS-PAGE12.5% (m v^{-1}), and the Hg concentrations were determined by graphite furnace atomic absorption spectrometry (GFAAS). **Results and Discussion** The gels showed great diversity of bands, with anaverage of 21.95 ± 3.66 bands per sample. In the control group, the presence of mercury was not identified. In mercury mapping of the lactating women from the Madeira River, we identified mercury in 12 bands, ranging from 2.62 ± 0.05 to 15.15 ± 0.53 mg $kg^{-1}$of Hg. This result is possibly due to the increased consumption of fish by the lactating residents compared to those in Goiânia, meaning a greater exposure to Hg. Besides the large amount of mercury released in the Amazonian environment because of its use in gold prospecting, several studies have indicated that the region is naturally rich in mercury, which contributes to higher Hg concentrations in the fish in the area, and consequently in the population, corroborating the results presented here. **Conclusion:** The SDS-PAGE electrophoresis was efficient in protein fractionation of human milk samples, as was the GFAAS to determine total mercury in protein bands. We did not identify Hg in the control group samples, possibly due to eating habitsand the history of Hg in the region. However, 12 bands from thesamples from lactating women residing along the Madeira River had Hg, which makes them possible candidates to be biomarkers for mercury toxicity.

OM 07- A TOXICOGENOMIC APPROACH FOR DEVELOPMENT OF BIOMARKERS OF PESTICIDE EXPOSURE

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Introduction: Northern Patagonia is an irrigated area holding 95% of exportable production of apple and pear. Around 900 ton/year of pesticides have been applied since year 2000 where 90% correspond to organophosphates. 50% of the pesticides applied are dispersed into the atmosphere without reaching the intended targets and ending mainly in irrigation canals, ponds, streams and rivers. Biomonitoring of environmental impact of pesticides using native species is a preferred resource for its ecological significance. *Rhinella arenarum* is a toad widely distributed in Argentina and less in Brazil, Bolivia and Uruguay. Organophosphates produce lethal and sublethal organism effects including presence of abnormalities, impaired growth and biochemical effects. In this sense, enzymes and metabolites of detoxification system are widely used as biomarkers. However, many of them are not specific and in several cases not responsive enough. Therefore, it is necessary to develop early, specific and sensitive ecotoxicological biomarkers. Here, we present a high throughput approach (RNA-Seq) to screen new potential biomarkers in *R. arenarum* larvae exposed to chlorpyrifos (CLP) pesticide. **Objective:** to develop new biomarkers for chlorpyrifos exposure. **Materials and Methods**:*R. arenarum* embryos were obtained by in vitro fertilization. Larvae (complete operculum (CO) + 11 days) were exposed to sublethal concentration of CLP (0.1 mg/L) [96h-LC50 2.5 mg/L)]. Samples were taken at 6h and 24h to evaluate reduced glutathione (GSH) levels and the activities of Glutathione S transferase (GST) and Catalase (CAT). At same time, samples were collected for RNA purification and massive sequencing. **Results and discussion:** GSH levels and GST and CAT activities were not significantly affected by CLP exposition neither 6h nor 24h compared with control. However, the RNA-Seq profiles showed differences between 6h vs 24h and compared to control. There was a first wave of gene expression at 6h where protein of several of them could drive transcription of genes detected at 24h. As expected, detoxification and oxidative stress pathways were activated. Beside, clustering of genes using Gene Ontology classification showed hits for Biological Process category: development, metabolic and cellular process, among others. **Conclusion:** biomarkers are a biological response to toxic exposition which can be used for management and environmental protection. Here, we show that classic biomarkers were no modified when toad larvae were exposed to sublethal concentration of CLP. However, using RNA-Seq several gene activation even at early time (6h) were detected. Thus, new molecular biomarkers could be defined and a combination of them could bring a more sensitive and specific tool as biomarker.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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OM 08- GRANDISIN INDUCES APOPTOSIS IN MULTIDRUG RESISTANT K562 CELLS

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Introduction: Chronic myeloid leukemia (CML) is a clonal myeloproliferative syndrome characterized by the chimeric gene *BCR-ABL*, whose product is a tyrosine kinase that contributes to apoptosis resistance. Currently, the CML treatment is TKI (tyrosine kinase inhibitors)-based therapy. However, second and third generations of TKIs were introduced in therapeutics due to drug resistance and toxicity of TKI first generation. In this context, natural compounds have been investigated as potential sources for development of drugs against various pharmacological targets, including leukemias. The tetrahydrofuran lignan grandisin, isolated from *Virola* and *Piper* species, presents antimalarial and trypanocidal. In addition, grandinsin also revealed antitumor properties, *in vitro* and *in vivo,* against Ehrlich Ascites Tumoral (EAT) model.
Objectives: In this work, the potential antileukemic activity of grandisin, a lignan extracted from *Piper solmsianum*, was evaluated against K562 cells, a multidrug resistant leukemic line.
Materials and Methods: The cytotoxicity of grandisin (0.018 to 2.365 µM) was evaluated in K562 and normal peripheral blood lymphocytes by Trypan Blue exclusion and MTT methods following 48h of drug exposition. Cell cycle analysis and apoptosis induction parameters were studied by flow cytometry and colorimetric assays.
Results and Discussion: K562 and normal lymphocytes were exposed to grandisin for 48 h and cell viability was evaluated by Trypan Blue and MTT assays. In both methods, cellular viability was concentration dependent and the IC_{50} values were lower than 0.85µM. Analysis of K562 cells after treatment with grandisin showed that the cell cycle was arrested in G1 phase with a 12.31% increase, while both S and G2 phases decreased. Furthermore, K562 morphological changes suggested an apoptotic process, which was confirmed by anexin V stain and caspase activation.
Conclusions: The lignan grandisin showed antileukemic activities against K562 cell line and the cell death process occurred via apoptosis.

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OM 09- EVALUATION OF DNA DAMAGE AND EXPRESSION OF GENES INVOLVED WITH HYPERTENSION IN NORMOTENSIVE AND HYPERTENSIVE RATS EXPOSED TO VITAMIN D3 DEFICIENCY

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Introduction: Vitamin D3 is a lipophilic micronutrient obtained from the diet (fatty fishes or fortified foods) or by the conversion of 7-dehydrocholesterol into previtamin D3 on epidermis after exposure to UVB radiation. Previous studies indicate that vitamin D3 deficiency occurs in 25% to 50% of the human population, and has been associated with hypertension development in normotensive individuals.
Objective: The aim of this study was to evaluate whether diets with different concentrations of vitamin D3 (control or deficient) would modulate DNA damage and regulate genes involved with hypertension pathways in spontaneously hypertensive rats (SHR) and their normotensive control Wistar-Kyoto (WKY).
Materials and Methods: During 12 weeks-treatment, SHR and WKY rats were fed AIN-93M (AIN-93 maintenance) diets, differing on the vitamin D3 levels: control diet (1,000 IU/kg diet) or deficient diet (0 IU/kg diet). DNA damage was evaluated in renal and blood tissue by comet assay and micronucleous test, and gene expression was assessed in renal tissue by RT^2 Profiler^{TM} PCR Array.
Results and Discussion: Our results showed that vitamin D3 deficiency induced high micronucleus frequency in the bone marrow and peripheral blood of SHR rats, and increased DNA breaks in peripheral blood of SHR and WKY rats. Regarding the expression profile of genes associated to hypertension, in both SHR and WKY rats, vitamin D3 deficiency increased the expression of gene *Ace,* involved in the pathway of the renin-angiotensin-aldosterone system. In SHR rats, vitamin D3 deficiency also increased the expression of more four genes of renin-angiotensin-aldosterone pathway (*Agt*, *Agtr1a*, *Agtr1b*, *Ece1*), five genes of smooth muscle contraction pathway (*Acta2*, *Cacna1c*, *Ednra*, *Kcnma1*, *P2rx4*), two genes of ion transport pathway (*Scnn1g, Slc7a1*) and one gene of vasoconstriction pathway (*Alox5*).
Conclusions: Vitamin D3 deficiency induced DNA damage and regulated genes involved with hypertension in normotensive and hypertensive rats. Vitamin D3 deficiency showed a more pronounced effect on hypertensive animals, inducing DNA and chromossomal damage, and regulating several genes involved with renin-angiotensin-aldosterone system and smooth muscle contraction pathways in SHR rats. The increase of DNA breaks in blood tissue of WKY rats showed that vitamin D3 deficiency also had a negative effect in normotensive rats.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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OM 10- POTENTIAL PROTEIN BIOMARKERS OF MERCURY TOXICITY IN AMAZONIAN FISH

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Introduction: It has known that the population of the Amazon region has to the base diet of fish, with consumption of about 200g of fish per day. Amongst the species consumed in this region, the carnivorous alimentary habits are most harmful for the population is having bigger levels of mercury accumulated in its organism. However, the fish of the base of the alimentary chain also present considerably high levels of Hg in his tissues. Studies show that fish of different ecosystem can reach different levels of methylmercury (MeHg) with toxicological relevance, even in areas considered unaffected by anthropogenic sources.
Ain: This study presents mercury quantification results in muscle and liver tissue samples from fish Jaraqui (*Semaprochilodus sp.*) and Curimatã (*Prochilodus nigricans*) collected in the areas of influence of the Jirau Hydroelectric Plant (UHE) - River Basin Madeira.
Materials and Methods: To analysis were made pools of liver tissue and other tissue and muscle followed a fractional precipitation using ethanol/chloroform and ethanol/hydrochloric acid yielding two pellets (proteins >90 kDa and <90 kDa). Both protein pellets molar mass >90 kDa and <90 kDa of the liver and muscle tissues were digested in ultrasonic bath using concentrated acid (H_2SO_4/H_2O_2), the Hg quantified by atomic absorption spectrometry graphite furnace (GFAAS) and proteins characterized by mass spectrometry ESI/MS.
Results and discussion: The spots that showed Hg had their characterized proteins resulting in 8 proteins, 2 muscle tissue of Jaraqui (Hemoglobin subunit beta and parvalbumin beta) and 5 liver tissue (parvalbumin-2, parvalbumin beta, Ubiquitin-40S ribosomal protein S27a, Keratin type II cytoskeletal 39S ribosomal protein 8 and L36 mitochondrial) and 4 of muscle tissue of Curimatã (parvalbumin-2, parvalbumin beta, parvalbumin alpha and Ubiquitin-40S ribosomal protein S27a). Between the two species, the Jaraqui had the highest concentrations of Hg in the samples studied: muscle and liver tissue (132±4 and 426±6 $\mu g\ kg^{-1}$ respectively) muscle and liver pellet <90 kDa (86±1 and 277±4 $\mu g\ kg^{-1}$ respectively) and Curimatã: muscle tissue (118±3 $\mu g\ kg^{-1}$), muscle pellet 78±0.9 $\mu g\ kg^{-1}$. The results were validated using the default certificate fish protein dorm 4-NCR containing 410±55 $\mu g\ kg^{-1}$ (408±3 $\mu g\ kg^{-1}$). The protein pellets molecular weight >90 kDa showed no mercury in any sample.
Conclusions: Despite Hg levels in the tissues of these fish does not find much higher than accepted by the FAO/WHO (0.5 mg/g), the daily consumption can cause serious health risks to the population who consume them, taking into account the process bioaccumulate in the tissues of this element.

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OM 11- METALOPROTEOMIC SPECIATION OF MERCURY IN AMAZONIAN FISH SAMPLES – MADEIRA RIVER BASIN

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Introduction: Mercury, especially in the last century, was widely used in gold mining in the mining regions of the brazilian amazon. These regions, mostly, were located in riverbeds or near them, significantly contributing to the contamination by mercury in aquatic animals. The Hg can bind to active sites of metalloproteins and paralyze them, preventing them from performing their function in the body, thus can take the animal to the internal imbalance or even death in severe cases of poisoning. **Aim:** Thus, the work seeks to optimize analytical methods for the application of metalomic in the development of possible mercury toxicity biomarkers in samples of muscle and liver of fish collected in the area of influence of the Hydropower Plant JIRAU – Madeira River, Rondônia, Brazil. **Materials and Methods:** The samples of muscle and liver of "Filhote" fish (*Brachyplatystoma filamentosum*) were macerated in an aqueous medium and the protein fraction present in the extracts, fractionated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).
Results and Discussion: The mapping of mercury in protein spots was done by atomic absorption spectrometry graphite furnace, with 87.40 ± 0.90 $m.kg^{-1}$ of Hg in muscle tissue, 1044 ± 13.60 $m.kg^{-1}$ in liver tissue, 72.20 ± 0.94 $m.kg^{-1}$ in muscle tissue pellet molecular weight <90 kDa and 867.60 ± 11.10 $m.kg^{-1}$ in liver pellets <90 kDa. The pellets molecular weight> 90 kDa showed no Hg. These spots that had Hg were characterized by ESI-MS 8 which resulted in proteins: glycolipid transfer protein, 40S ribosomal protein-Ubiquitin S27a, Betaine - homocysteine S-methyltransferase 1, Macoilin-2, N-terminal Xaa-Pro- Lys N-methyltransferase 1, 1 GTP cyclohydrolase feedback regulatory protein, characterized in liver tissue and parvalbumin beta 1 GTP cyclohydrolase feedback regulatory protein, transmembrane protein Ubiquitin-186 and 40S ribosomal protein S27a, in muscle tissue. **Conclusion:** From the results obtained by quantitation by GFAAS can observe a considerable amount of Hg in these tissues, highlighting the liver tissue with a larger amount of Hg. The protein fractionation by 2D-PAGE proved to be a good alternative for protein separation and identification of potential biomarkers of Hg in amazon fish.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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ALTERNATIVE METHODS TO ANIMAL EXPERIMENTATION

A | R | T
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AM 01- CURCUMIN ATTENUATES OXIDATIVE DAMAGE AND IMPROVES LEARNING AND MEMORY IN RAT MODEL OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease (AD) is one of the most common forms of progressive neurodegenerative diseases. The progressive deficits of learning and memory are a key clinical hallmark of AD, and increasing evidence suggests that oxidative damage contributes to AD pathogenesis. Curcumin shows diverse bioactivities, such as excellent in anti-oxidation and anti-inflammation.
Objective: In the study, to investigate the protective effects of curcumin on the oxidative damage and abilities of learning and memory on a rat model of AD.
Methods: In AD model group, rats (250-300 g) were injected into bilateral hippocampus with 1.0 mg/bw of streptozocin (STZ) and after 7 days continuously injected intraperitoneally with 125 mg/bw/day of D-galactose for 60 days. For curcumin-protective group, rats that injected with STZ and D-galactose were intraperitoneally injected with 10 mg/bw/day of curcumin for 60 days. The abilities of learning and memory were evaluated based on the assay of Morris water maze before execution. Rats were executed and biomarkers indexing oxidative stress were assessed, including malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) in serum and glutathione (GSH) in liver homogenate.
Results: Model rats show a higher oxidative damage compared with normal rats (7.4±1.3 *vs* 6.1±1.8 nmol/L MDA, 262±32 *vs* 340±33 U GSH-Px, and 2.2±0.3 *vs* 2.6±0.2 mg GSH/g prot). However, rats in curcumin-protective group decreased oxidative damage compared with model rats alone (6.9±1.3 *vs* 7.4±1.3 nmol/L MDA, 316±34 *vs* 262±32 U GSH-Px, and 3.0±0.7 *vs* 2.2±0.3 mg GSH/g prot). Further, Model rats spent more time to find out the platform under the surface of water in Morris water maze compared with normal control (40.3±1.8 *vs* 24.9±1.6 s). However, rats in curcumin-protective group could find the platform more quickly than in AD model group (36.8±2.1 *vs* 40.3±1.8 s).
Conclusions: Curcumin improves the ability of AD model rats treated with hippocampal injection of STZ and intraperitoneal injection of D-galactose on learning and memory. Curcumin decreases oxidative damage in AD model rats. These results imply that curcumin performs the biological function of neuroprotective activity.

Keywords: Alzheimer's disease; Curcumin; D-galactose; Neuroprotection; streptozocin

AM 02- THE UTILITY OF QSARS IN PREDICTING ACUTE FISH TOXICITY OF PESTICIDE METABOLITES: A RETROSPECTIVE VALIDATION APPROACH

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Introduction: Under EC Regulation 1107/2009 there is a requirement for registrants to establish whether pesticide metabolites are potentially harmful to the environment, and as such fish acute toxicity assessments may be carried out. The number of metabolites can be considerable; thus this area of testing may use many vertebrates. EFSA's recent "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" outlines opportunities to apply non-testing methods such as Quantitative Structure Activity Relationship (QSAR) models. Provision of a scientific evidence base to support the use of QSARs in the prediction of the acute fish toxicity of pesticide metabolites, and subsequent regulatory acceptance, could reduce the numbers of animals used.
Objective: The aim of this work is to examine the potential for QSARs to be used for pesticide metabolites, through a retrospective validation approach.
Materials and Methods: Ecotoxicity data for 679 metabolites contained within the Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm) were extracted and filtered to restrict the dataset to metabolites with experimental fish LC50 values. QSAR calculations were performed for 190 metabolites using the US EPA's ECOSAR software. The most conservative LC50 value generated by ECOSAR was selected for comparison to experimental LC50 values. The relationship between the predicted and experimental values was assessed.
Results and Discussion: Preliminary analysis revealed a significant correlation between predicted and experimental fish LC50 values ($r = 0.60$, $p < 0.001$, Spearman correlation). For the majority of the metabolites the predicted values were equal to or lower than their experimental values. For the remaining values the mean ratio of experimental:predicted LC50 was 0.24 (SEM 0.03), i.e. QSAR predicted values were on average only 4.1 times higher than those derived experimentally. Considering normal experimental variability, and the risk assessment procedure, these estimates may have regulatory utility.
Conclusions: This initial conservative analysis indicates that there is value in further refining the QSAR approach to improve the prediction of acute fish toxicity of pesticide metabolites. Refinements to this analysis will be undertaken before this method can be considered for incorporation into regulatory guidance.

A | R | T
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AM 03- DEVELOPMENT OF AN *IN VITRO* EPIDERMAL MODEL TO EVALUATE SKIN SENSITIZATION

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Introduction: Nowadays, several assays involving animal testing are used for the evaluation of immunotoxic effects such as immunosuppression and sensitization. However, they present secondary issues such as high costs, ethical issues and doubts regarding relevance to human risk assessment. The development of novel *in vitro* methodologies are promising tools to overcome the problems involved in animal testing and to successfully predict risk assessment. Currently, the main restrictions of *in vitro* skin models are the absence of an immunocompetent commercial kit and the scarce development of new models. Different *in vitro* models were validated to assess the corrosion or skin irritation (eg, SkinEthic™, EpiDerm™, EpiSkin™). In contrast, to other parameters such as the potential for sensitization, there are no fully validated *in vitro* models and its development is an immediate and important challenge for the cosmetic industry. **Objective:** The aim of this work was to develop an immunocompetent epidermal model capable of evaluating skin sensitization of chemicals. **Materials and Methods:** The present work was conducted in three steps: 1) development of a functional epidermis equivalent (EE) (according OECD GD 439); 2) use of THP-1 cell line to discriminate sensitizers (4-nitrobenzilbromide, resorcinol and eugenol) from non-sensitizers (DMSO); 3) generation of an immunocompetent epidermis equivalent (IEE) by the addition of immune cells (THP-1) to the EE system. The evaluation of the IEE functionality was held by testing the potential to distinguish skin sensitizers. **Results and Discussion:** The EE developed in this study presents organized skin layers observed by microscopy, viability and barrier functions according to guide 439 from OECD. The EE developed responded well to quality parameters such as histology analysis, viability and barrier functions according to guide 439 from OECD, also, irritants were able to be distinguished from non-irritants. Through the evaluation of the expression of CD86, CD54 and the release of cytokine IL-8, THP -1 cells in monolayer were able to distinguish sensitizer agents from non-sensitizers. The incorporation of THP-1 cells in the EE resulted in an efficient cross talk that permitted the development of a complete IEE. This model was more efficient to distinguish sensitizers from non-sensitizers when compared to THP-1 cells in monolayer determined by the expression of the CD86 and CD54. IL-1α showed to be a good parameter to distinguish sensitizers from non-sensitizers better than IL-8 but it was not able to rank the potency of the sensitizer. **Conclusions:** We concluded that our model is highly relevant and likely to be used to evaluate skin sensitizers.

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AM 04- DEVELOPMENT OF SKIN AND EYE EPITHELIA AS ALTERNATIVE METHODS TO ANIMAL USE IN COSMETICS TESTING

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Introduction: Toxicity evaluation is essential for determining the damage that a substance may cause to the human body by different exposure routes. For many years, animal models have been used to evaluate ingredients and/or products toxicity. However, a strong discussion has stimulated the scientific community to provide alternative methods for the assessment of toxicological endpoints, especially for cosmetics evaluation. Several methodologies have already been implemented, but it represents a complex topic that goes beyond technical development including the issue of regulatory acceptance. In Brazil, the National Council for Animal Experiments Control (CONCEA) has recently approved a list of 17 alternative methods to replace or reduce the number of animals used in toxicological tests, whose application shall be mandatory from 2019. **Objective:** To establish *in vitro* alternative methods, focused on skin and eye epithelia, for the safety and efficacy evaluation of cosmetic ingredients and/or products. **Materials and Methods:** Human cells were commercially obtained, including skin keratinocytes, melanocytes and retinal cells for epithelial formation; and skin fibroblasts for complementary full skin development. Cell number, culture media and time of differentiation in the air-liquid interface were optimized for each model. Histological evaluation was carried out with hematoxylin-eosin and/or Fontana-Masson staining. **Results and Discussion:** Different tissues were developed, including non-pigmented and pigmented cutaneous epidermis, full-thickness reconstituted skin (including dermis), and retinal epithelium. Histological analyses showed that epithelial differentiation was effective in all models, comparable to literature patterns and similar to human tissues. Differentiation time varied between 7 and 21 days in air-liquid interface, according to each experimental model. As expected, the total number of epithelial layers was higher in skin epidermal structure, and retinal epithelium did not present stratum corneum. The addition of melanocytes did not change the pattern of epidermal formation, while the presence of dermal structure showed to increase the amount of epidermal layers suggesting an interactive communication between the two skin compartments. **Conclusion:** *In vitro* methods represent a powerful tool for cosmetic products evaluation and distinct models might be considered for different exposure routes and also for the achievement of complementary results. The present work was based on the analysis of skin and eye irritation, but further studies should be certainly addressed for different approaches, such as efficacy assessment and generation of innovative claims.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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AM 05- INTEGRATED SAFETY STRATEGY FOR DEVELOPMENT OF CHILDREN COSMETIC PRODUCTS USING *IN VITRO* METHODOLOGIES

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Introduction: Brazil is one of the largest cosmetic markets and represents great opportunities for several beauty niches, including children's products. Regarding safety assessment and considering the particular needs of the target market, children's products must be specifically formulated and require special attention in order to avoid inappropriate use and adverse reactions. Since animal tests are no longer accepted for cosmetic evaluation in Europe, the major challenge in this field is to ensure reliable products using alternative methods and available data in literature. **Objective:** To define an integrated theoretical and technical rationale for suitable development of children's make-up products (lipstick, gloss, blush and nail polish). Without applying animal methods, formulation safety analysis, toxicological *in vitro* tests (cytotoxicity, phototoxicity and skin irritation) and clinical trials were considered. **Material and Methods:** Firstly, a systematic study was performed for selection of the intended ingredients that could be used in each formulation. *In vitro* methods were applied for the evaluation of cytotoxicity, phototoxicity (following the internationally validated guidelines OECD129 and OECD43, respectively), and acute skin irritation (using reconstituted human skin model based on OECD439). Complimentary clinical trials were conducted on adults and children, under pediatrician supervision, for the assessment of skin irritability, sensibility, photoallergy, phototoxicity and tolerability in real conditions of use. **Results and Discussion:** Theoretical component of the rationale for children's products evaluation was based on: 1) simple formulations with fewer ingredients in comparison to products for adults; 2) raw materials properly analyzed according to their chemical structure, level of exposure and toxicological profile, including available literature; and 3) fragrances within IFRA recommendations. Concerning *in vitro* results, none of the children's products was classified as significant cytotoxic, phototoxic or skin irritant in the tested concentrations. Clinical trials also showed negative results for all the toxicological endpoints analyzed, considering adult and children's panels.
Conclusion: The safety rationale developed in the present work, using specific formulation criteria and *in vitro* alternative methods to animal use, showed to be assertive and well correlated to the results of clinical trials. It represents a practical, integrated and valuable tool for the development of appropriate formulations and the safety assessment of children's cosmetic products.

AM 06- TOXICITY OF MALE NATURAL LATEX CONDOMS USING BIOLOGICAL ASSAYS: A CONTRIBUTION TO ACTIONS OF SANITARY SURVEILLANCE

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INTRODUCTION: The development of research products for use mainly for health protection of the population is considered of utmost importance especially when it encompasses Sanitary Surveillance. These include materials manufactured from latex of natural rubber that may come into contact with the human body. As examples of these we may cite male natural latex condoms made of impervious material from latex of natural rubber with the purpose of preventing the passage of body fluids during sexual intercourse as well as help preventing pregnancy, sexually transmitted diseases and the HIV virus. Natural rubber did not have much use until the discovery of the vulcanization process by Charles Goodyear. The vulcanization agents which are more often used as system components for the process of rubber vulcanization are: sulfur, thiazoles like the 2-mercaptobenzothiazole and its derivatives, carbamates and thiurams. The production of both domestic and imported male condoms available in Brazil meets strict criteria that cover from aspects of the latex quality to specifications for primary packaging, consumption and transportation. These products must be tested by reputable institutions (INMETRO) based on RDC 62/2008, which certify the quality of the product assuring the emission of the ISO 9002 quality standard. **OBJECTIVE**: Evaluating of the toxicity in male natural latex condoms using the *in vitro* cytotoxicity assay - agar diffusion method. **METHODOLOGY:** Thirty seven (37) samples of both domestic and imported male condoms of different brands were analyzed by the *in vitro* cytotoxicity assay - agar diffusion method. **RESULTS:** A total of thirty seven (37) samples were analyzed, we verified that 24 (64.9%) presented toxicity level 2 (low); 12 (32.4%) presented toxicity level 3 (moderate); 1 (2.7%) presented toxicity level 4 (severe) and none presented toxicity level 0 (absence). **DISCUSSION:** Due to the fact of the continually growing range of healthcare products and an important nonconformity incidence mainly in the analysis of male condoms, it´s necessary that an evaluation of their biological safety for possible toxic substances detection be made by employing *in vitro* assays instead of *in vivo* with laboratory animals. **CONCLUSION:** As a result, the study of possible toxic effects induced by these healthcare products must receive the attention of Sanitary Surveillance in order to minimize and prohibit their use as much as possible, in case their cytotoxic effects have been proven.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AM 07- TOXICITY OF BDE-100 (REPRESENTATIVE OF BROMINATED FLAME RETARDANTS) IN DIFFERENT CELL LINES

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Brominated flame retardants are used in a wide array of polymer-containing products and polybrominated diphenyl ethers (PBDEs), more precisely the BDE-100 congener, is one of the most widely used representatives of this class. Thus, human exposure to BDE-100 through various routes poses deleterious health effects. Endocrine disruption was one of the first described toxic effects of these compounds, and some reports in the literature show toxicity in several organs. However, the mechanism of action of this particular compound is not well described. This study characterizes the *in vitro* toxicity the BDE-100, assessed in different cell lines using monolayer (2D) and tridimensional cell culture by hang drop (3D cell culture) attempt to elucidate the mechanism of toxicity in different cell lines. HepG2 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin, in 5% of CO_2 at 37 ^{o}C and plated in 12-well plate for adhesion or in 96-well hanging drop plate (Insphero) for 3D cell culture. Hepatocytes were isolated from healthy Wistar rats; after cannulating the portal vein, the liver was perfused with Krebs-Henseleit buffer at 37 ° C gassed with a mixture of 95% O_2 and 5% CO_2. HeLa cells were maintained in the same conditions of HepG2. All cell lines were exposed to BDE-100 (0.1-25µM). After 24 and 48 hours of exposure to BDE-100 cell viability was assessed using 0.5% MTT and the formazan crystals formed were solubilized and correspondent absorbance was assessed at 570 nm wavelength. In addition, the determination of mitochondrial membrane potential ($\Delta\psi$) was performed using the fluorescent probe TMRM, and also the total protein/cell mass was evaluated using the SRB assay. Finally, we assessed the damage caused by BDE-100 in HepG2 3D-cultures using the fluorescent dyes Hoechst (nuclear morphology) and Ethidium (membrane integrity) and inspecting the cells using a Nikon Eclipse TS100 microscope. BDE-100 has been shown to cause cellular dysfunctions by dissipating $\Delta\psi$ and decreasing cell viability and cell mass in all cell lines cultured in monolayers. Additionally, it was observed an increase in the number of cells labeled with ethidium in HepG2 3D-culture, also indicating cell death. We concluded that the exposure to BDE-100 induces significant damage in several cell lines tested, both in the immortalized and in primary cells, and demonstrated to induce cell death in 3D culture too. This damage corroborate with the induction of cell death in HepG2 in monolayer and effects may be related to the ability of the compound to induce mitochondrial damage as previously ascertained (Pereira et al., 2013)

Reference: Pereira et al., Basic & Clinical Pharmacology & Toxicology, 112, 418-424.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AM 08- BOVINE CORNEA OPACITY AND PERMEABILITY TEST (BCOP) PREDICTION FOR EYE IRRITATION OF AGROCHEMICAL FORMULATIONS

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Introduction: Eye irritation is an acute toxicity endpoint addressed when registering agrochemical formulations. The *in vivo* Draize rabbit eye test (OECD 405) is the regulatory accepted test for the determination of the full range of eye irritation potential. Alternative toxicological methods have meanwhile gained importance because of animal welfare (reduction, refinement, and replacement of animal testing), improved toxicological knowledge during product development and shorter time to market.
Objective: The present study aimed to assess the predictive capacity of the *in vitro* eye irritation Bovine Corneal Opacity and Permeability (BCOP) test of agrochemical formulations, compared to the *in vivo* Draize method. The two different classification systems, United Nations Globally Harmonized System of Classification and Labeling of Chemicals (UN GHS) and Brazil ANVISA toxicological classification (Port.03/1992), have been compared.
Material and Methods: A total of 111 agrochemical formulations were tested *in vitro* (BCOP) and compared to existing *in vivo* data, being 97 performed at BASF SE (Germany) and 14 at TECAM Laboratory (Brazil). The laboratories followed the same protocols, internationally validated: OECD TG 437 and OECD 405 for BCOP and Draize rabbit test, respectively. The data from both labs was analyzed together, the formulations Brazil ANVISA classes II, III and IV were grouped together for the analysis; the same for US GHS categories 2A, 2B and unclassified.
Results: The 21 formulations assigned to UN GHS category I were also assigned to Brazil ANVISA class I, however 44 additional formulations were also assigned to Brazil ANVISA Class I. Specificity of the BCOP test was 39% for UN GHS and 61% for Brazil ANVISA, considering *in vitro* irritation score (IVIS) < 3. Sensitivity for the most severe categories was very low for both UN GHS (5%) and Brazil ANVISA (5%). The overall accuracy was 32% for UN GHS and 28% for Brazil ANVISA classification system.
Discussion and Conclusion: Based on the same *in vivo* eye irritation data, Brazil ANVISA classification ended up in more Class I products compared to UN GHS. BCOP results were associated with high number of false negatives in both UN GHS and Brasil ANVISA classification systems, and thus underpredicted the eye irritation potential. The applicability of the *in vitro* method should be evaluated (also depending on the classification system) to define how to improve prediction. Strategies for combining different *in vitro* assays to improve prediction are being investigated.

Key words: eye irritation, alternative methods, Draize method, BCOP, agrochemical formulations.

AM 09- USE OF MONOCYTE ACTIVATION TEST (MAT) AS ALTERNATIVE METHOD FOR RABBIT PYROGEN TEST (RPT): HEMATOLOGICAL PROFILE AND EVALUATION OF INTERLEUKIN-1B RELEASE FROM CRYOPRESERVED BLOOD STIMULATED WITH ENDOTOXIN

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Introduction: Currently, there are three testing possibilities to evaluate pyrogen contamination: the Rabbit Pyrogen Test (RPT), the Limulus Amebocyte Lysate test (Bacterial Endotoxin Test), and test systems using human whole blood or human monocytes, called Monocyte Activation Test (MAT). The MAT is based on the human fever reaction and thus most closely reflects the human situation. This test can be used cryopreservation of pooled human blood for detection of cytokine response to endotoxin. Therefore, it is important to evaluate the influence of cryopreservation processing and storage of blood in this response. **Objective**: To evaluate the effects of cryopreservation on blood cell counting and cytokine response between individual and pooled fresh blood and cryopreserved blood during 4 months. **Metodology**: Heparinized whole blood pool of 4 health donators was drawn by venous puncture. The complete blood count was performed using an automatic hematology analyzer (Hemogram 60 BioClin®). LPS response was monitored on fresh and cryopreserved pooled blood from single and multiple donors. The release of IL-1b was quantitated fresh and after storage (7, 15, 30, 60, 90 and 120 days) by ELISA. **Results and discussion**: A decrease in the number of total cryopreserved leukocytes was observed from the seventh day. When results were analyzed by the different cell types, the percentage (but not the absolute number) of monocytes showed a slight increase, while the percentage of granulocytes decreased during the freezing days. Regarding the functionality of monocytes/lymphocytes, results showed that there was no statistically significant difference ($p \leq 0,05$) in reactivity (IL-1b) of the cryopreserved for fresh blood during the 120 days. The use of blood pool tends to equalize the results. **Conclusion**: It was shown that the cryopreservation process and storage did not affect the absolute number of monocytes/lymphocytes or their ability to respond to endotoxin stimuli.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AM 10- ALTERNATIVE METHODS ON ANIMAL USE: THE PROCESS OF VALIDATION

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Introduction: Since 2005, OECD published the Guideline number 34 that establishes de procedures for validation and international acceptance of methods. The Decree 6,899/2009 states that an alternative method must be validated and internationally accepted. The Law 11,794/2008 created the Council for Controlling Animal Experimentation (CONCEA) which is the responsible of becoming official an alternative method in Brazil. Nowadays, Brazil counts on three entities responsible for the validation process: 1) Brazilian Center for Validation of Alternative Methods (BraCVAM); 2) Network of Alternative Methods (RENAMA); and 3) CONCEA. They are independent, not hierarchically linked but each of them play the specific role in the process. **Objective:** To show the validations process of an alternative method to be adopted in Brazil. **Methods:** All three may propose to validate a method, but, BraCVAM is responsible for organizing the study together with RENAMA, preparing the final report and recommend the assay to CONCEA. RENAMA is composed by laboratories that execute the assays and CONCEA, based on BraCVAM's report, will become the method official. In case of a new validation different groups are envolved: a) Validation Manager Group (VMG) which is responsible for all decision of the process; b) Observers for technically and legally helping VMG; c) Selection, Coding and Distribution of substances; d) Statistics. Validation is performed by a Leader Laboratory and, at least, two Participating Laboratories. In the case of already internationally validated methods, BraCVAM directly recommends CONCEA the recognition of them. **Results and Discussion:** In 2015, Brazil is performing the validation of HET-CAM (Hen's Egg Test – Chorion Allantoic Membrane) with the participation of international experts on validation process and on the test. Representatives of ECVAM, OECD, MAPA and ANVISA are participating as Observers. The Lead Lab is from France and four Brazilian labs are participating. HET-CAM is being proposed as a test to be used together with already validated BCOP, in order to constitute a battery of assay to replace rabbits in the Draize Eye irritation Test. This process, with the help of international experts, may lead Brazil to be part of countries that are contributing to the replacement of animals around the world. **Conclusion:** The Mutual Acceptance of Data that Brazil signed with OECD and the BraCVAM participation on ICATM may contribute to the recognition of validation performed in Brazil.

AM 11- BRAZILIAN STUDY FOR THE VALIDATION OF THE HEN'S EGG TEST - CHORIOALLANTOIC MEMBRANE (HET-CAM)

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The validation of the Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) is the first validation study conducted in Brazil. This process is one of the strategic actions, which are taking place within the scope of the Brazilian Network of Alternative Methods (Renama) and counts with the Brazilian Center for Validation of Alternative Methods (BraCVAM/ INCQS) as the coordinator of the validation study. It has four main purposes: (*i.*) To conduct a validation study to assess independently the reliability (reproducibility intra- and inter-laboratories) and relevance (predictive ability) of the HET-CAM to identify chemicals not requiring classification for eye damage/irritation, combining the experimental results with existing HET-CAM data; (*ii.*) To complement international efforts to develop testing strategies for eye damage/ irritation; (*iii.*) To build capacity within Brazil and BraCVAM on the conduct of validation studies of *in vitro* methods for use in a regulatory context; and (*iv.*) To familiarize national regulatory authorities on alternative methods, their validation and their potential use in a regulatory context. The following activities have been conducted so far: definition of the Validation Management Group (VMG), first meeting of the VMG and first training of the participating laboratories (N=5) on the study protocol. The study will take place in two main steps. A first preliminary study has the purpose to measure the difficulties occasionally found by the participating laboratories and, if necessary, to proceed elucidations and/or adaptations of the study protocol. The second step will be conducted by the laboratories, which presented satisfactory results on Step 1, and is intended to confirm - in an enlarged number of chemical samples - the validity of the method according to a final version of the study protocol. The validation process will be conducted in accordance with OECD Guidance Document 34.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AM 12- THE NATURAL DYE ERYTHROSTOMINONE DOES NOT INDUCE ORGAN-SPECIFIC GENOTOXICITY TO 3D SKIN EQUIVALENT AND HEPG2 CELLS

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Introduction: Synthetic dyes are extensively used in many industries, such as cosmetic, textile, pharmaceutical and food. Since the synthetic dyes induce toxic effects, as allergies and mutagenicity, natural dyes have been used to develop safe and eco-friendly consumer products, in order to replace harmful synthetic dyes used in manufactures, particularly in cosmetic applications. Once humans might be exposed to dyes through dermal contact with consumer goods (e.g. cosmetics), genotoxicity studies are needed to estimate their hazard in a context of organ-specific effects. Due to the 3R principles (replacement, reduction and refinement) for animal testing, *in vitro* alternatives have been developed to toxicological assessments, comprising different cell culture systems (2D and 3D). The choice of each culture system depends on the complexity of the *in vivo* system that intends to reproduce *in vitro*. Hepatogenotoxicity assessment has been usually performed with hepatocyte monolayer cultures (2D), whereas skin equivalents (SE), an organotypic culture (3D), can closely mimic the structure and physiology of natural skin. Considering the target organ of cosmetic industries, the SE is a suitable model for assessing genotoxic effects of dermally applied chemicals, such as dyes. Even though, secondary organs have to be considered, once dyes can penetrate through the skin and reach non-target organs through blood vessels, such as the liver. **Objectives:** The cyto- (MTT test) and genotoxicity (Comet assay) of the natural dye erythrostominone, a red product obtained from a fungu, were assessed using SE and human hepatoma cells (HepG2).**Materials and Methods:** SE [immortalized human keratinocytes (HaCaT), normal human dermal fibroblasts (NHDF)] and HepG2 were cultured and further exposed (MTT test: 24 hr; Comet assay: 3 hr) to the natural dye in the range of 35 -522 mg/L to HepG2 and 10-500 mg/L to SE, negative (SE: acetone; HepG2: DMSO) and positive controls. The cell viabilities were measured by spectrophotometry. DNA breaks were analyzed by fluorescence optic microscopy using the Comet IV software, randomly measured by the tail intensity. **Results and Discussion:** The erythrostominone dye did not induce cytotoxic effects to SE, whereas induced to HepG2 at concentrations from 435 mg/L as dose-dependent manner. This different response of cytotoxicity between SE and HepG2 probably may be due to the different metabolic activities of each cell type and the tested models. We did not observe genotoxic responses for any of the tested concentrations of the dye to both tested system, even at high concentrations. **Conclusion:** According to our findings, we concluded that the natural dye erythrostominone is a promise alternative for the use of synthetic dyes for cosmetics proposes.

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AM 13- TESTING CYTOTOXIC ACTIVITY OF ANTIDIABETIC PLANT ACHILLEA SETACEA WALDST. & KIT. USING THE MTT ASSAY

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Introduction: *Achillea* species are widely used in folk medicine in central Anatolia region in Turkey. *Achillea setacea* Waldst. & Kit. is one of those species used mainly as emmenagogue, as well as in the treatment of stomach ache, menstrual disorders and diabetes. However its cytototoxic activities have never been tested previously. **Objective:** To investigate the potential antidiabetic activity; search cytotoxic effects and help to develop regulatory guidelines for a safe consumption. **Materials and Methods**: The hypoglycemic effects of aqueous and ethanol extracts of *A. setacea* were evaluated primarily with *in vivo* methods in normal, glucose loaded hyperglycemic and streptozotosin-induced diabetic rats. To understand the antihyperglycemic mechanism, α-glucosidase and α-amylase enzyme inhibitory effects of the plant extracts were investigated *in vitro* and Acarbose was used as a reference. The cytotoxic activities of the extracts were determined through MTT assay, against human cervical carcinoma cell lines (HeLa) and human liver carcinoma cell lines (HepG2). All data were expressed as the mean ± SEM and analyzed by ANOVA. $P \leq 0.05$ was considered statistically significant. **Results and Discussion:** Our findings with *in vivo* and *in vitro* experiments support the traditional usage of the plant against diabetes. Results indicated that blood glucose levels of STZ-induced diabetic rats were decreased by *A. setacea* ethanol extract at dose of 500 mg/kg compared to control group (6.44-30.23%). Moreover ethyl acetate fraction obtained from ethanol extract by solvent-solvent extraction, showed significant antidiabetic activity (14-24%). Ethanol extract showed promising α-glucosidase enzyme inhibitory activity with 57.61 and 21.96% at doses of 3000 and 1000 µg/ml respectively. On the other hand *A. setacea* aqueous extract was found to be non-toxic at even highest dose of 3000 µg/ml concentration.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AM 14- TOXICITY EVALUATION OF THE NOVEL PHOTOPROTECTIVE COMPOUND LQFM048 SYNTHESIZED THROUGH GREEN CHEMISTRY APPROACH

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Introduction: The new heterocyclic derivative LQFM048 (2,4,6-tris ((E)-ethyl 2-cyano-3-(4-hydroxy-3-methoxyphenyl)acrylate)-1,3,5-triazine) was originally designed through molecular hybridization strategy from Uvinul® T 150 and (E)-ethyl 2-cyano-3-(4hydroxy-3-methoxyphenyl)acrylate sunscreens, using green chemistry approach. This compound presented global yields of 78%, interesting redox potential and thermal/UVA stability. Since LQFM048 showed an increment of 64% in the photoprotection in relation to ethylhexyl metoxycinamate standard sunscreen, this compound has been considered a promising candidate to a novel photoprotective. **Objective:** To evaluate the safety of the LQFM048 using predominantly alternative methods. **Materials and Methods:** Eye irritation was evaluated by Short Time Exposure (STE), Bovine Corneal Opacity and Permeability (BCOP), Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) and hemolytic assay was performed using mice erythrocytes. Skin toxicity was investigated by MTT assay and IL-18 secretion in HaCaT keratinocytes, and Local Lymph Node Assay (LLNA):BrdU-ELISA. The i*n vitro* 3T3 neutral red uptake phototoxicity assay and micronucleus assay were also carried out. **Results and Discussion:** In eye irritation assessment, LQFM048 was non-cytotoxic in STE test and did not promote changes in the corneal permeability, opacity and histology. Additionally, presence of hemorrhage, vessel lysis and/or coagulation was also not detected in HET-CAM assay as well as non-hemolytic profile in erythrocytes. In skin toxicity evaluation, LQFM048 presented non-phototoxic in 3T3 cells, non-cytotoxic and did not change IL-18 secretion response in keratinocytes. In LLNA:BrdU-ELISA, hexyl cinnamic aldehyde and eugenol positive controls showed a stimulation index (SI) of 2.4 and 1.9, respectively, being classified as sensitizers. By the other hand, LQFM048 was considered as non-sensitizer (SI=0.7). Additionally, no clinical signs were observed in the mice. Moreover, LQFM048 did not show potential mutagenic. **Conclusion:** The new photoprotective compound LQFM048 showed safe for endpoints investigated and its use in cosmetic and pharmaceutical products with sunscreen property is promising. Moreover, the multiparametric platform obtained here seems interesting in the toxicity evaluation of new compounds.

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AM 15- MIXTURE OF INGREDIENTS USED IN PERMANENT HAIR DYES LEADS TO MORPHOLOGICAL CHANGE, INCREASES CELL DEATH AND DNA FRAGMENTATION IN *in vitro* EPIDERMAL RECONSTRUCTS

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Introduction: Permanent hair dyes are the most representative class among hair dyes reaching up to 80% of worldwide consumption. These dyes are formed after successive reactions between a primary intermediate (ex. *P* phenylenediamine) and a coupler (ex. Resorcinol) that after oxidative reactions with hydrogen peroxide (H_2O_2) results in permanent color changes inside the hair shaft. In 2001, the European Union began re-evaluating the toxicity of hair dyes, and since 2007, 85 hair dyes have been banned for not being considered safe for consumers. According to the European Union there is the need to investigate the toxic potential of these ingredients using more modern techniques as most data available are from 1970s and 1980s. **Objective**. The purpose of this study was to evaluate the toxic potential of some ingredients and their mixtures used in permanent hair dyes using an *in house* developed epidermal equivalent model. **Methods** Epidermal equivalent were incubated with 2000 mg/mL of *p*-phenylenediamine (PPD), 2000 mg/mL of Resorcinol and 2% of H_2O_2 alone and combined among them for 24 hours, next, cell viability was performed using MTT reduction assay. Morphological aspects of Epidermal equivalent were evaluated with Hematoxylin and eosin (H.E) and also immunofluorescence assay were used to detected DNA fragmentation using TUNEL method. **Results and Discussion:** Our results regarding MTT reduction assay show that 2000 mg/mL of *p*-phenylenediamine (PPD), 2000 mg/mL of Resorcinol and 2% of H_2O_2 did not significantly reduce epidermis viability. Although, the combination of PPD/ H_2O_2 and PPD/Resorcinol/ H_2O_2 reduced cell viability to around 80%. Moreover, we observed morphological changes and increase of DNA fragmentation in epidermis treated with the mixture of the ingredients when compared to each ingredient alone and negative control.
Conclusão: The findings provide the evidence that the mixture of ingredients decreased cell viability, resulted in cellular morphological changes and induced DNA fragmentation in higher levels when compared to ingredients alone and negative control. Considering that epidermis is the first route of exposure to hair dyes, we suggest that similar effects could be induced in human skin after exposure to hair dyes. In addition, we recommend the use of epidermal reconstructs for toxicity screening tests of permanent hair dyes.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

AM 16- IN VITRO SKIN IRRITATION ASSAY OF MEDICAL DEVICES IN THE CONTEXT OF ISO 10993-10

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Skin irritation is one of the 3 toxicological endpoints that are always assessed for medical devices. Yet, even if the ISO 10993-10 standard mentions the in vitro OECD test guideline (OECD TG 439), the reference test method remains the in vivo model. The objective of this poster is to present an *in vitro* protocol on a Reconstructed Human Epidermis (SkinEthic RHE) to assess skin irritation of medical devices. SkinEthic RHE model is validated in the TG439 as a full in vitro replacement method to assess the skin irritation of chemicals. Yet, TG439 concern neat chemicals and is not adapted to medical device extracts in which potential leached irritants are diluted in a solvent. The proposed protocol is closed to the one developed for cosmetics products with a long exposure time, 24 hours instead of 42 minutes for pure chemical, and no post-incubation (compared to 42 hours for pure chemicals). The benchmark of this protocol has been done according to the publication of Casas et al.1 on polar and apolar solvents spiked with irritants and on different medical devices extracts. The ability of the model to detect low concentration of known irritants spiked at different concentrations in polar and non-polar solvents. The results show a dose response toxicity of lactic acid into PBS and of Heptanoic acid into Sesame Oil. The second part of the work presents the results of the test performed onto sample of polymers, PVC and silicone, used for medical devices. Some of these samples, specifically produced for this experiment contain known irritant chemicals. These polymers were prepared according to ISO 10993-12, Biological evaluation of medical devices – Part 12 (Sample preparation and references materials) and extracted in a polar (PBS) and a non-polar (Sesame Oil) solvent. Cell viability, Il-1 release and histology have been studied 24h after treatment. The results show the ability of this in vitro method to detect low concentration of irritant in solvents used for medical devices extraction. This in vitro method is also able to discriminate between medical devices containing irritant or not. With the set of samples tested, a cut off of 50% of cell viability was sufficient to do the classification. These preliminary data suggest that cell viability alone could be a sufficient biological endpoint to measure for medical devices classification (Cell viability, Il-1a release and histology have been studied 24 hours after treatment). The transferability of the protocol have been confirmed with a parallel study in an independent US laboratory. The ongoing confirmation of these results in a round robin study with more samples and more laboratories could lead in the near future to an evolution of the ISO 10993-10 guideline for medical devices to replace the in vivo skin irritation Draize test by *in vitro* testing on human reconstructed epidermis.

AM 17- RECONSTRUCTED HUMAN EPIDERMIS (RHE): FROM SKIN IRRITATION TO SKIN SENSITIZATION

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Releasing a new product to the market is a costly and long process for drug, chemical or cosmetics. Early prediction of human health hazard is important to avoid termination of promising candidates in latest stages. According to some surveys (Olson 2000, Greaves 2004), classical pre-clinical approaches based on animal studies exhibit weaknesses to predict some human toxicity. Of all tissues, skin shows the least concordance (36%) between effects in animal and human. This underlines the need for more predictive *in vitro* approach. Moreover, regulations such European directives (2003/15/EC for cosmetics, 2010/63/EU on animals used for scientific purposes and EC 1907/2006 for Registration Evaluation Authorization of Chemical substances) promote the use of alternative to animal testing for industry. Limits of animal models and regulatory developments strengthen the need for robust alternative models to the animal. Episkin develops and markets reconstructed human epidermis models for research, toxicological and pharmacological evaluation. These models constructed from primary human cells reproduce the histological, morphological, biochemical and physiological properties of human epidermis. They allow you to get rid of the animal in predictive toxicology for various endpoints, either for screening or regulatory. In pharmacology, their proximity to human tissue in vivo make them tools of knowledge and powerful evaluation. This presentation will discuss methods and results of studies to assess several toxicological endpoints with RHE models from skin corrosion/irritation to skin sensitization. Skin corrosion/ irritation data are essential to any toxicological file and have long been based on the animal. Today, validated methods with reconstructed human epidermis replace the use of animals for the classification of chemicals. In July 2013, new versions of the OECD guidelines for skin irritation (TG439) and skin corrosion (TG431) have been released. Skin sensitization is a more complex toxicological endpoint as shown by the adverse outcome pathway (AOP) initiated by covalent binding to proteins. In the sequentially identified events the step of keratinocyte activation leads to the development of different assays using RHE models. These promising assays based on IL_18 quantification or transcriptomic studies (SENS-IS method) could be part of future integrating testing strategies to predict human skin sensitization. The *in vitro* reconstructed human epithelia models reproduce the main features of human *in vivo* tissues. Compared with cell 2D cultures, the 3D organization of these models reproduces more realistic test conditions. Their robustness, reproducibility and proximity to targeted human tissues make it possible to overcome the animal to build *in vitro* screening architectures and predictive assessment of the effects in humans. They are approved for certain regulatory tests (skin corrosion/irritation) and are also used in many toxicological (penetration, phototoxicity, genotoxicity ...) and pharmacological protocols. Moreover they have evidenced time and cost savings. For all these reasons in vitro reconstructed human tissue models are massively used for safety and efficacy screening.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FOOD TOXICOLOGY

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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FT 01- DETERMINATION OF CIANYDE IN FRESH TAPIOCA STARCH COMMERCIALIZED IN NATAL/RN BY MOLECULAR ABSORPTION SPECTROPHOTOMETER

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Introduction: During cassava flour production, the roots from the species *Manihot esculenta* are milled and starch is extracted from this procedure, originating the tapioca starch, major ingredient used during tapioca preparation. Tapioca is a food very appreciated and commonly consumed by population from North and Northeast Brazil regions. It is served accompanied by different kinds of stuffing. Recently, the consumption of tapioca is increasing and getting very popular also in the other regions of Brazil as a carbohydrate alternative source in diets without gluten. Cyanogenic glycosides protect vegetable species from predators due it ability to liberate hydrocyanic acid and cyanide. It is known that cassava is a food source that presents cyanogenic glycosides. **Objective:** The aim of the present study was determine the concentration of cyanide in tapioca starch. **Methods:** Six different tapioca starch blends commercialized in supermarkets in Natal/RN were obtained. Cyanide quantification was conducted by a colorimetric method applied to two distillated portions obtained from 20g from each sample. Before distillation the samples were submitted to an acid hydrolysis during four hours. Then, 125 mL of a first distillated portion was collected, inside an erlenmayer containing, initially, 29mL of sodium hydroxide 2.5%. A second distillated portion were collected (125 mL) before substituting the first erlenmayer to another one, also containing initially, 29mL of sodium hydroxide 2.5%. An aliquot of 5mL of each distilled fraction was transferred to tube reactions. Alkaline picrate solution 0.5% was added in each tube at a volume of 5mL. Cyanide reduces alkaline picrate resulting in an orange to red complex with absorbance measured at 490nm. The molecular absorbance of each distillated fraction was measured by spectrophotometer (490nm) and employed for cyanide concentration calculation. **Results:** A calibration curve, with correlation coefficient (R^2) approximately as 1.0, was obtained with standard solutions containing 10, 25, 50, 75, 100 and 125 µg cyanide/mL. The methodology used detected 114.65, 165.23, 207.30, 339.38, 296.98 and 348.33 ppm of cyanide in the samples analyzed. **Conclusion:** All samples presented cyanide concentrations above 50 ppm, limit stipulated to classify fresh vegetables as toxic or not. The cassava starch is used for the preparation of tapioca. The baking procedure promotes reduction of cyanide levels.

Uniterms: Cyanide, hydrocyanic acid, tapioca, *Manihot esculenta*, toxicity.

FT 02- HEALTH RISK ASSESSMENT OF CHEMICALS – A COMMENTARY ON REQUIREMENTS FOR THE PROVISION OF TRAINING.

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Training programs on chemicals and health risks exist within different European Organisations and Universities, but currently there are no agreed European standards on the training of chemical health risk assessors. The need for practical training has been recognised in meetings on need for risk assessment training organized by DG SANCO (DG SANCO, 2007, 2008). Data collected in the market study "Preparation of a mapping of existing courses relevant to the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and the classification, labelling and packaging (CLP)" available from universities, other academic institutions and professional organizations within the European Economic Area (EEA) commissioned by the European Chemicals Agency (ECHA) to the University of Milan, support the limited number of comprehensive full courses available throughout Europe offering the necessary training requirements in human health risk assessment (University of Milan, 2012).

The requirements for the provision of training in the field of human health risk assessment of chemicals draw on the experiences gained from many training initiatives throughout Europe, for example training qualifying for EUROTOX European Registered Toxicologist (ERT) and Guidance on Risk Assessment Advanced Training Programme (RAAP), the EU-funded-projects: European Toxicology Risk Assessment Training (TRISK) and Risk Assessment and Management – European Training Programme (Risk Assets). Despite such interest, however, it appears that a comprehensive training course that truly focuses on all steps in the risk assessment process and provides both theoretical and practical training is not harmonized throughout Europe.

Assuming all goes well and the EN 16736 "Health risk assessment of chemicals - Requirements for the provision of training" will be approved in a final formal vote, a European standard will be available and adopted in late 2015 by all CEN member states. The draft standard specifies that ideal courses in health risk assessment of chemicals should be multidisciplinary and cover toxicology, epidemiology, exposure assessment, risk characterization, ethics and quality control and implications for risk management and risk communication. Also applied training with hands on experience such as case studies or examples of concrete risk-assessments should be part of these courses.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FT 03- TOXICITY OF FERULIC ACID ON CHINESE HAMSTER OVARY (CHO) CELLS AND THE UNDERLYING MECHANISM

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Introduction: Ferulic acid (FA) is a phenolic acid that is ubiquitous in plants and has many health benefits, but its potential toxic effects need to be investigated thoroughly prior to its application in healthcare products. Therefore, establishment of a safety evaluation system for plant active substances such as FA is urgently needed. **Objective:** The present study investigated the toxic effects of FA on cell survival, mitochondrial function and genomic integrity. The relationship of FA toxicity with multiple signaling pathways was also explored to obtain detailed insights into the toxicology of FA. **Materials and Methods:** Chinese hamster ovary cell line was purchased from cell resource center of institute of basic medical science Chinese academy of medical science center. Colorimetric CCK-8 assay was used to determine the effects of FA on the cytotoxicity of CHO cells. Cell apoptosis and cell cycle were detected by annexin-V/PI assay by using flow cytometer. Comet Assay was used to assess the number of DNA breaks. Micronucleus rates were analyzed by the micronucleus module of the IN Cell Analyzer 1000. Mitochondrial Membrane Potential, ATP release, reactive oxygen species (ROS was analyzed using flow cytometer. The proteins expression was measured by western bolt assay. SPSS 18.0 software was used for statistical analysis. **Results and Discussion:** This study found that high dose FA can inhibit the survival of CHO cells, with the half maximal inhibitory concentration (IC_{50}) being about 1600 μM. At concentrations of 800 μM-1600μM, FA caused cell apoptosis and cell cycle arrest. Results of comet assay and micronucleus test also showed evidence of genetic toxicity of high dose FA. Investigation of the mechanism underlying these damages revealed that 800μM-1600μM FA caused increase in intracellular ROS, which then activated the NF-κB pathway and upregulated the expression of the apoptosis executor caspase-3 and the pro-apoptotic protein bad. However, the toxic effects of FA were found to be unrelated to the PI3K/Akt pathway. Increase in expression of the DNA repair protein rad51 was observed, which indicated that DNA damage had been induced by high dose FA. **Conclusion:** High concentration of FA caused elevation in the ROS level in CHO cells while activating the NF-κB and the caspase pathways. Furthermore, FA up-regulated bad protein in a PI3K/Akt independent manner. Through mitochondrial damage and genotoxicity, FA led to significant decline in cell viability, increase in the apoptosis rate of CHO cells, and inhibition of cell proliferation. This study is the first to systematically examine the potential toxic effects of FA and the underlying mechanism, which will provide a basic foundation for future application of FA in food and healthcare products.

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FT 04- DETERMINATION OF ESSENTIAL (Ca, Fe, Mg, Mn) AND TOXIC (As, Cd) ELEMENTS IN BRAZILIAN TEA HERBS AND ITS INFUSIONS BY ICP-MS

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Introduction: Tea is one of the most consumed beverages in the world and offers multiple benefits, due to its high content of phenolic compounds and essential elements. In contrast, also contains some undesirable trace elements that may be harmful to health. The metals contained in herbs are extracted differently in infusions, first because of intrinsic factors of these plants, and second because people do not respect a standard procedure in the tea infusions preparations. **Goal:** We evaluated how much of some essential (Fe, Ca, Mg, Mn) and toxicity (Cd, As) elements found in teas herbs common used in Brazil are extracted during the infusion process. Also, was assessed if this extraction depends on infusion time and bustle of infusion. **Materials and methods:** Were analyzed teas of boldo (*Pneumus boldus Molina)*, chamomile (*Metricaria recutita* L.), lemon grass (*Cymbopogon citratus* Stapf) and mint (*Mentha piperita* L.) marketed in Brazil. To determine the total content of essential and toxic metals present in the herbs, about 100mg of each plant species were exactly weighed and subjected to an acid digestion assisted by microwave, followed by determination of metals by ICP-MS. To evaluate the fraction of essential and toxic metals that were extracted during the preparation of tea infusions, infusions teas were prepared varying time of contact between the sachet and water (5 or 10 minutes), and the agitation or not of the infusion, simulating everyday situations and enabling the determination of the concentration of the extracted metals. After that, the tea infusions also were analyzed by ICP-MS. **Results and discussion**: In general, potentially toxic chemicals elements have not been extracted for the herbs analyzed. In turn, essential elements showed a high extraction, with the exception of manganese, which may be of interest, since in this metal might be toxic in high levels. The most extracted elements in lemon grass and mint teas were Mg and Ca, while in boldo were Mg and Fe. The extraction of the mint and the lemon grass teas varied as extraction time; in relation to time and agitation extraction, Cd showed no significant difference in any of the samples, as also, the elements present in chamomile teas showed no significant differences. The estimate of the average contribution of consumption of teas to the daily intake requirements of the chemical elements will be further present. **Conclusion:** It concludes that the extraction varies according to the species of herb, showing that additional studies should be conducted regarding metal bioavailability in tea infusions.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FT 05- INFLUENCE OF RICE CULTIVARS (Oryza sativa L.) IN ARSENIC AND CADMIUM MITIGATION AFTER CULINARY PROCESS

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Introduction: Rice (Oryza sativa L.) is an important component of the Brazilian diet and is the second largest cereal crop in the world. It accumulates considerable amounts of essential elements, but also toxic elements such as, arsenic (As), and cadmium (Cd). **Objectives**: Evaluate different rice cultivars obtained under the same growing and soil conditions regarding the retention of As and Cd and determine the loss in the washing and rice cooking steps, how, in fact, the population is exposed. Moreover, there is such cultivars are in accordance with the ceilings set by Anvisa in RDC nº 24/2013 and the Codex Alimentarius. **Materials and methods**: The analyzed samples were provided by Embrapa Clima Temperado and culinary preparation was performed in laboratory according RAAB1 with modifications. After that, samples were freeze-dried and subjected to acid digestion assisted by microwave according to the method proposed by NARDI2. Isotopes analyzed by ICP-MS were 75As and 111Cd. To check the accuracy of the analytical method was used Rice Flour SRM 1568a. The results were expressed as mean ± standard deviation. Data were analyzed with an analysis of variance (ANOVA), and a test of the means Tukey's test at a 5% level of probability. The estimated daily intake was calculated according to the formula IDA = Ceq x M and compared to PTDI. **Results and discussions**: Only two cultivars are in agreement with the RDC 24/2013, which concentrations were below 0.30 mg/kg of As. Regarding Cd, all of them were consistent to RDC 24/2013, been below to 0.40 mg/kg. Washing rice with water before cooking reduced the arsenic concentrations in raw rice by 3 to 11%, depending on cultivar. For arsenic, the culinary preparation could significantly reduced the concentration in most cultivars. However, three of the seven analyzed cultivars did not have significant difference on As mitigation after culinary preparation. Arsenic daily intake is within the allowed by FAO/WHO, and the analyzed rice cultivars were contributing about 1.75 to 15.86% of the PTDI into arsenic intake. **Conclusion**: One of the cultivars which is still being developed by Embrapa and CIRAD was considered the safest rice cultivar due to low concentration of As and Cd in raw grains. Furthermore, this cultivar had a considerable loss for the culinary preparation. The cultivars BR Irga 409 and BRS Sinuelo CL spite of the high concentration of As in raw rice, were the cultivars that had greater influence of washing and baking, respectively, for the loss of As. In general, culinary preparation was considered effective in mitigating the As.

Bibliography:

1 J Environ Monit:11, 41, 2009.
2 Food Chem: 112, 727, 2009.

FT 06- EFFECT OF JUICES FROM AMAZON FRUITS ON THE ENZYMATIC ACTIVITY OF HUMAN P-GLYCOPROTEIN IN VITRO

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Introduction: Juices from different fruits are concomitantly administered together with medicines on routine basis. However, this association has been recently linked to drug-nutrient interactions since some juice components may be able to interact with drugs during absorption process. The main pharmacokinetic mechanisms related to these interactions are transport and metabolism carried out by some proteins located in enterocytes, such as P-glycoprotein and cytochrome P450 enzymes, respectively. Considering that there is a great variety of juices from amazon fruits regularly consumed by the local population, these beverages may have a potential to alter drug action due to the effect on P-glycoprotein.
Objective: Assess the effect of juices from amazon fruits on the enzymatic activity of human P-glycoprotein.
Materials and Methods: Therefore, juices of fruits popularly consumed were selected in this investigation as follows: biribá (*Rollinia mucosa*), cubiu (Solanum sessiliflorum), cupuaçu (*Theobroma grandiflorum*), manga (*Mangifera indica*) and umari (*Poraqueiba sericeia*). The fresh fruits were juiced and diluted to the concentration of 50 ug/mL. The processed samples were evaluated for flavonoids and total phenols content and for enzymatic activity using the luminescence-based "P-gP-Glo™ Screening Systems", which employs human recombinant P-glycoprotein. Statistical analyses were performed utilizing GraphPad Prism 6.
Results and discussion: Low contents of flavonoids and total phenols were observed in these samples (0.4-0.7%) and (2.8-3.0%), respectively. Biribá, cupuaçu, manga e umari samples presented inhibitory action on P-glycoprotein in vitro. On the other hand, cubiu had no effect on this enzyme in the conditions assayed.
Conclusions: According to the results obtained, these samples may have the potential to inhibit P-glycoprotein in vitro. However, further studies should be done to confirm these findings and to clarify the mechanisms involved in this phenomenon.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FT 07- BRAZIL NUT "MILK" TOXICOLOGY: INFLUENCE OF SPRAY DRYING CONDITIONS ON THE AFLATOXIN AND SELENIUM CONTENT

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Introduction: the health-related properties described to some functional foods can be derived from natural constituents of these foods or addition of ingredients that modify original properties. Therefore, industries are seeking different production methods to meet this consumer demand for healthier and natural products. The use of a drying method, known as "spray drying", for example, has allowed the development of new foods. Thus, various raw materials from the Amazon region have been tested for new products. Among them, is the Brazil nut, with recognized protein and lipid content and excellent source of selenium (Se) as antioxidant. The Brazil nut is ingredient for several products, including the "milk". However, their natural content of selenium (Se) can be toxic in high doses. Moreover, it may be associated with the presence of carcinogenic metabolite aflatoxin (AFL). In that sense, it is necessary to know the nutritional and toxicological aspects of new products, beyond the nutritional and sensory characteristics.
Objective: the characterization of the Brazil nut "milk" was carried out in order to evaluate toxicological aspects concerning selenium (Se) and aflatoxin (AFL) content.
Material and Methods: the tests were a factorial design of the type 2^2, with independent variable the type of drying aid (maltodextrin and arabic gum). As dependent variables were the technological characteristics of the extracts and the proportion of adjuvants. The Se content was evaluated according to Olson et al. (1975) and the AFL was carried out according to AOAC (2005).
Results and Discussion: Among the different tested spray drying conditions, the selected product had 30 % Arabic gum and 16 % yield. However, the process conditions raised the level of AFL. After processing, the raw material that previously met the legal limits for shelled Brazil nut with 4.0 mg/kg, increased to 65 mg/kg. The same was observed for Se content. After processing, the product with better yield was 1.200 g of Se/100g. As the consumption of a tablespoon (10g), diluted in 100 ml, will be 120 µg of Se, we can consider the value above the acceptable RDA 55 mg/day for adults.
Conclusion: the spray drying process in Brazil nut "milk" seems to concentrate, in toxic levels, compounds such as Selenium and Aflatoxin. It is important to test the raw Brazil nut previous to the processing, to avoid contaminants and meet the legal standards for food safety. More effort is necessary to study other adjuvants or temperature/pressure of atomizer to combine an attractive yield and nutrients of the product for the industry.

FT 08- EFFECT OF BLANCHING ON THE CONTAMINATION OF FRENCH FRIES BY 3-MONOCHLOROPROPANE-1,2-DIOL FATTY ACID ESTERS

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Introduction: Fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD esters) are processing contaminants that can be formed in refined vegetable oils and fats at significantly high concentrations. As a result of the frying process using contaminated oil, fried foods may represent an important source of these contaminants in the diet. Dietary exposure to 3-MCPD esters has been considered a priority food safety issue since free 3-MCPD can be released through the action of gut lipases, representing a public health concern in view of its toxic properties.
Objective: In order to identify potential mitigation strategies to reduce consumer exposure to 3-MCPD esters, the objective of this study was to evaluate the influence of blanching on the levels of these compounds in French fries.
Materials and Methods: The experiments were carried out using potatoes (*Solanum tuberosum* L.), cultivar Asterix, acquired in the local market. The potatoes were peeled, cut out in length of the center of the potato (cross-section of 1 x 1 cm and length of 5-10 cm), and blanched before frying at 85 ºC/5 min and at 98 ºC/3 min. A non-blanched sample was used as control. The samples were fried in palm olein containing 2.86 mg/kg of 3-MCPD esters. The mean initial temperature and time of frying were 182 °C and 6.5 min. Fried samples were analyzed in relation to fat uptake and 3-MCPD esters. The contaminants were determined by an in-house validated indirect method based on acid transesterification and gas chromatography-mass spectrometry.
Results and Discussion: The fat uptake (%) after frying was 8.31±0.25 for the non-blanched control sample, 11.05±0.91 for the sample blanched at 85 ºC/5 min and 9.05±0.74 for the sample blanched at 98 ºC/3 min. The levels of 3-MCPD esters (mg/kg) were, respectively, 0.20±0.00, 0.27±0.02 and 0.22±0.03. As can be observed, blanching as applied in this study increased fat uptake and, consequently, the contamination of the product by 3-MCPD esters, especially when it was carried out at lower temperature and longer time (85 ºC/5 min).
Conclusions: The contamination of French fries by 3-MCPD esters may be influenced by pre-treatments such as blanching. Good correlation was observed between the levels of the compounds in the final product and fat uptake. As blanching increased fat uptake and 3-MCPD esters contamination up to 33%, this treatment could not be suggested as a potential mitigation strategy to reduce consumer exposure to these contaminants.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FT 09- SPECTROPHOTOMETRIC DETERMINATION OF SODIUM NITRITE IN MEAT PRODUCTS

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Introduction: The market of meat products has shown great expansion and high competitiveness in the last decade, since the consumption of meat products like sausages, fresh sausage and mortadella has become in creasingly present in food habits of the population. The curing salts, such as nitrate and sodium nitrite, are widely used as food additives in the processing of meat products and are intended to conserve, enhance or modify the sensory properties of food. However, several studies have shown that nitrite intake in high amounts can be potentially hazardous to human health, due to the action of nitrosamines in the human body, may cause mutagenic, carcinogenic, neurotoxic and nephrotoxic effects, and also induce methemoglobinemia. Although the Ministry of Health, in its Decree No.1004/1998, establishing a maximum concentration of these preservatives in foods, 150 $mg.kg^{-1}$ (sodium or potassium nitrite) and 300 $mg.kg^{-1}$ (nitrate sodium or potassium), meat products such as mortadella, sausages and fresh sausages, often have higher nitrite content as established by law.
Objectives: This study aims to quantify, through the spectrophotometric method, sodium nitrite content in mortadella, sausages and fresh sausages, marketed in the region of Avaré/SP, and compare the results with the values recommended by the legislation.
Materials and Methods: The samples were acquired from commercial establishments in the city of Avaré/São Paulo and region. The meat products were used mortadella, sausage and fresh sausages. Three brands of each type of meat product were analyzed and two replications performed on each sample in order to obtain the medium and more accurate results. The analytical classic method used was spectrophotometry in 540 nm. 288 assays were performed. Statistical analysis of the Anova complemented by the Tukey test was used to compare means.
Results and Discussion: The mean nitrite concentrations in meat product ranged from 29.25 $mg.kg^{-1}$ to 249.80 $mg.kg^{-1}$. Statistical results of the Anova showed a highly significant difference between different brands of meat products analyzed ($p<0.0001$). Among the meat products, the fresh sausage brands A and C showed a nitrite content above the permitted by Brazilian law (maximum 150 $mg.kg^{-1}$).
Conclusion: Based on this information, it is concluded that the fresh sausage has high nitrite content and that government agencies in Brazil need to monitor more strictly the addition of nitrites in meat products.

FT 10- CONCENTRATION OF CADMIUM, LEAD AND ARSENIC IN CHILDREN'S FOOD RICE BASE

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One of the most consumed food worldwide is rice (*Oryza sativa L.*), behind only wheat. It is estimated that the 2014/2015 global rice production is around 470 million tons (milled basis), according to *Foreign Agricultural Service* (FAS-2015). Rice provides energy (carbohydrates) has an easy absorption and shows low allergic potential. Important source of nutrients for children where its derivatives are the first solid food ingested. Rice highlights from other foods, not only because of its nutritional value, but also has a certain predilection to uptake and accumulate arsenic (As), lead (Pb) and cadmium (Cd). These elements are widespread in the environment, either naturally in rocks, in water, minerals or by human action, through mining, industrial processes or pesticides. The two group of species for As includes organic (As-o) and inorganic (As-i). The compounds of As-o, such as arsenobetaína, are commonly found in seafood and can be consumed normally. However As-I, such as As^{+3} and As^{+5}, can cause serious health damage. Exposure to low concentrations of As, Pb or Cd over time can cause skin cancer, lungs, neurological disorders, cardiovascular diseases and diabetes. Due to these factors children show a high risk to these elements. Thus, rice-based foods for children were analyzed. Porridge and baby food were selected from different countries such Brazil, Germany, Canada, Mexico and Australia for determination of Pb, Cd and As. Foods were digested according to Batista et al. (2014). The determinations were performed by mass spectrometry with inductively coupled plasma (ICP-MS, Agilent 7900, EUA). The concentrations were i) baby food (n= 12) As: 74,99 ± 113,921 µg/Kg; Cd:175,05± 209,54 µg/Kg; Pb: 275,27± 504,10 /Kg; ii) porridge (n=13) As: 98,39± 47,98 µg/Kg; Cd: 13,64± 7,16µg/Kg; Pb: 28,63 ±13,12 µg/Kg. The World Health Organization (WHO-2010), estimates limits of ingestion by weight for As, Pb and Cd of 0,1-3; 0,02- 3; and 0,07-0,4 µg/kg per day, respectively. The values found in the present study show that there are high concentrations of these elements which can bring risks to the health of children at high intake. New parameters of food safety and processing for child are needed in order to improve the nutritional/toxicological risk for this special population.

References

1.B. L. Batista, M. Nigar, A. Mestrot, B. A. Rocha, F. B. Junior, A. H. Price, A. Raab and J. Feldmann. Journal of Experimental Botany. 65, 1467-1479 (2014).
2.U.S 2015/16 Rice Plantings Indicated at 2,92 Million Acres (USDA,USA,2015) http://www.ers.usda.gov/media/1821099/rcs-15d.pdf. Acessed in 17.07.15

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FT 11- FUMONISIN B1 AND OCHRATOXIN A MYCOTOXINS AND BIOMARKERS DETERMINATION IN ANIMAL TISSUES AND SERUM AS AN ASSESSMENT OF DIETARY EXPOSURE

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Introduction: Assessment of human and animal exposure to mycotoxins is usually performed by the analysis of foods, feeds and beverages. However, a better method of assessing exposure to mycotoxins is to measure tissues and biological fluids. This approach is advantageous because it helps in estimating mycotoxins intake in terms of quantity and quality. **Objectives:** The aim of this study is to determine the three major mycotoxins feed contaminants (Fumonisins B_1 and ochratoxin A), their tissues and serum biomarkers (sphingosine (So) /sphinganine (Sa)) as a way of assessing animal exposure. 203 samples (serum and tissues) were collected from pigs and analysed using HPLC. **Results:** The results obtained revealed that samples from rural and commercial farms were respectively contaminated with fumonisin (FB_1) in 85-87% of serum and 52-66% of tissues and OTA in 68-85% serum samples and 14-39% in tissues. Fumonisin B1 was found with a min 1.19 ppb in kidney samples followed by 0.69 ppb in liver and 3.4 ppb in muscle tissues while Ochratoxin A was found at 28.5 ppb in kidneys, 15.5 ppb in liver and 1.8 ppb in tissue muscles. **Results:** Analysis of sphingosine (So) /sphinganine (Sa), which are the fumonisin biomarkers, revealed that there was no correlation between incidence of FBs in serum (87%) and tissue (60%) samples compared to So /Sa occurrence in serum (45%) and tissues (40%). In addition, among the samples analysed (87%), the levels of FBs did not necessarily correspond to So/Sa in serum or tissues. This shows that there is no necessary association between FBs exposure and So (Sa) levels in tissue and serum as previously reported in some studies. It was also observed that not all FB_1 positive serum or tissues samples had proportional so/sa with higher So and lower Sa. The findings also contradict results obtained by Van der Westhuizen *et al*. (2008; 2010) who reported that there is correlation between Fumonisin B1 exposure and sphingosine (So) /sphinganine (Sa) in monkeys. Fumonisin B1 is mostly found in muscle tissues mycotoxin while ochratoxin A was noticed in kidney mycotoxins. **Conclusion:** The novelty of this study is that it reveals the ability to detect more than one mycotoxin in analysed samples but also raises issues of the effects of multi mycotoxins exposure for animals as well as humans consuming animal products. In addition, it confirms the fact that sphingosine (So) /sphinganine (Sa) are biomarkers for fumonisins but cannot be used for the quantification of fumonisins exposure in animals as previously thought.

FT 12- OPTIMIZATION AND VALIDATION OF ANALYTICAL METHOD TO DETERMINATE FLUOROQUINOLONES AND TETRACYCLINES RESIDUES IN BROILER CHICKEN MUSCLE

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Introduction: The presence of residues of antibacterial drugs in foods is an important public health problem and these substances may be present in food as a result of productive practices. Brazilian law establishes Maximum Residue Limits (MRLs) at 100 µg L^{-1} to fluoroquinolones (FQs - enrofloxacin, ciprofloxacin and norfloxacin), and 200 µg L^{-1} to tetracyclines (TCs - chlortetracycline, tetracycline, oxytetracycline and doxycyclin) in poultry muscle. **Objective:** In this present work, a method based on High Performance Liquid Chromatography coupled to tandem Mass Spectrometry (HPLC-MS/MS) was optimized and validated to determine FQs and TCs residues in broiler chicken muscle. **Materials and Methods:** The validation method followed the guides according to the documents "Guidance on validation of analytical methods" (2010) and "Manual of analytical quality assurance - residues and contaminants in food" (2011). Enrofloxacin_d5 and Demeclocycline were used as internal standards for FQs and TCs, respectively. The mobile phase used was water (A) and methanol (B), both acidified with 0.1% formic acid. Chromatographic separations were performed using a Zorbax SB-C_{18} column (50 x 4.6 mm; 1.8 µm) and a C_{18} column guard (4.6 x 12.5 mm; 5 µm), both from Agilent®. The muscle samples, previously triturated and homogenized, were weighted (2 g) and EDTA solution was added to avoid tetracyclines chelation. Samples were homogenized for 30 min with acetonitrile acidified. After, the samples were centrifuged for 10 min at 4000 rpm (4 °C) and the supernatant was kept under refrigerating (-18 °C) for 1 h. Thereafter, the samples were centrifuged, all solvent was evaporated under nitrogen flow, resuspended with water:methanol (70:30) and centrifuged again. The final supernatant was analyzed by HPLC–MS/MS. **Results and Discussion:** Good linearity (r^2>0.99) was obtained for all drugs achieved in concentrations from 0.0, 0.25, 0.50, 1.0, 1.5 and 2.0 x MRL levels. Intra and interday precision with relative standard deviation (RSD) lower than 8% were obtained at levels at 0.5, 1.0, and 1.5 x MRL levels, which was in agreement with specifications. Furthermore, the method showed average recoveries ranging from 99.8% to 100.3%, proving its accuracy. The limits of detection (LOD) for FQs was 1.5 and between 2.5 and 10 for TCs. The limit of quantification (LOQ) for FQs was 2.5 and between 5 and 15 for TCs. The values of parameters of decision limit (CCa) and detection capability (CCβ) were between 107,9 and 223,0 µg L^{-1} for CCa and 115,8 and 246,1 µg L^{-1} for CCβ. **Conclusions:** A simple and cheap extraction was optimized, providing a methodology fast and effective for routine laboratory work. The performance parameters of the method are accordingly with the guides used for the validation.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FT 13- RECENT DEVELOPMENT ON RISK ASSESSMENT FOR FOOD SAFETY IN SOUTH EAST ASIAN REGION (ASEAN)

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The food safety is one of the main concerns in ASEAN region and worldwide. For ASEAN, the organization under the ASEAN Expert Group on Food Safety (AEGFS) is in the process to harmonize the regulation and control among member states. In order to be able to draw the management scheme or option effectively and suitably for the control of food safety, the risk assessment is the key information for the risk management. At the regional level, the Framework on Monitoring and Surveillance developed under the ASEAN Food Safety Improvement Plan (AFSIP) of the AEGFS has identified ASEAN Member states (AMS) capacity building needs on risk assessment and has established a work plan. At the same time, the risk assessment activities are also being carried out by AMS individually. Regionally, the risk assessment center is in the process of establishment. The center is called ASEAN Risk Assessment Center (ARAC). The members agreed that ARAC should serve as a coordinating centre on risk assessment in ASEAN and it will be located in Malaysia, which will also provide secretariat support to ARAC. The coordination on the risk assessment activities among AMS is one of the main responsibilities of the centre. It may be possible that the risk assessment could be performed in a specific AMS as the network of ARAC. The progress is now in the process of darfting SOP for operation in various aspect.

At the national level in Thailand, National Food Committee Act in 2007 to coordinate the management of food activities including food security, food quality and safety, and food education. The strategic committee on food quality and safety is established to manage the food issues according to the strategic framework of food management approved by the cabinet in 2010. The sub-committee on risk assessment is established and it is proposed to establish an organization to coordinate and perform risk assessment called Thailand Risk Assessment Center (TRAC). This center operated jointly between National Food Committee and Mahidol Unversity to make sure its independency and autonomy are assured to avoid conflict of interest among the center and the food safety management authorities or ministries. The sub-committee proposed the organization of the center and it is in the process of producing guidelines for risk assessment composed of general guideline, guideline on chemical risk assessment (including food additives, contaminants, pesticide residues, veterinary drug residues and food contact materials), microbial risk assessment, and safety assessment of genetically modified food derived from recombinant DNA. The framework of the national risk assessment is drafted and approved. The work plan is laid down including collecting of expert roster in various area, the process of capacity building, and communicating among sakeholders.

Keyword: Food safety, Risk Assessment, Thailand Risk Assessment Center, TRAC, ASAN Risk Assessment Center, ARAC

FT 14- OPTIMIZATION OF A MULTI-MYCOTOXIN METHOD USING LC-MS/MS

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Introduction: Cereals such as maize, rice and wheat are staple foods all over the world, contributing to more than 60% of the worldwide energy intake. However, these commodities and their products may be contaminated by aflatoxins, fumonisins, ochratoxin, deoxynivalenol and zearalenone, mycotoxins potentially hazardous to human health. The toxicity of these mycotoxins is mainly related to their carcinogenic, nephrotoxic and immunotoxic properties.
Objective: The objective of this work was to optimize a single method for the analysis of aflatoxins (AFB1, AFB2, AFG1 and AFG2), citreoviridin (CTV), deoxynivalenol (DON, 3 AcDON, 15 AcDON, deepoxi-deoxynivalenol and deoxynivalenol-3-glucoside), fumonisins (FB1, FB2, FB3, HFB1), ochratoxin A (OTA), and zearalenone (ZON and α-zearalenol) in maize, rice and wheat using LC-MS/MS (4000 QTRAP – ABSciex).
Methods: The analyte-dependent MS/MS parameters were optimized by a direct infusion of mycotoxins solutions (200-800 ng/mL; dissolved in MeOH/H_2O, containing 1 mM ammonium format and 0.1% formic acid) into the mass spectrometer, at a flow rate of 10 µL/min. Ion source parameters were optimized using a 80 ng/mL D3G standard solution, at 0.8 mL/min. The analytical procedure was based on a solid-liquid extraction (SLE) using an ultrasonic bath, followed by centrifugation and filtration prior to injection. In order to define the best extraction conditions we compared three different solvent composition for the SLE procedure: ACN:H_20 (80:20) 0.1% formic acid, ACN:H_20 (80:20) and MeOH:H_20 (80:20). The SLE procedure was tested for maize flour, rice and wheat flour. Recovery tests were carried out in triplicates, for each solvent composition, at concentrations ranging from 2.2 to 224 µg/kg, depending on the analyte. Matrix matched standard curves were prepared between 0.96 and 1600 µg/kg. All mycotoxins were analyzed in the ESI positive mode.
Results: For all matrixes evaluated, the best results were obtained using acidified ACN as the extraction solvent. For rice, recoveries ranged from 78.9% (CTV) to 134.4% (FB3) and relative standard deviations (RSD) from 1.75 (ZON) to 16.1% (AFG2). Recoveries for maize flour ranged from 72.9% (CTV) to 239.2 % (FB2 – sample naturally contaminated) and RSD from 4.1% (ZON) to 35.6% (FB2). For wheat flour, recoveries ranged from 57.2% (FB1) to 115.8% (CTV) and RSD from 0.0% (ZON) to 27.2% (FB3).
Conclusion: The results obtained showed that the SLE with acidified ACN is suitable for the multi-mycotoxin method, being a rapid and cost effective extraction procedure. After validating the method, cereal samples will be analyzed and a dietary exposure assessment will be conducted.
Acknowledgments: CNPq, CAPES e FAP-DF.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FT 15- PRODUCTION OF HYDROLYZED FUMONISINS

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Introduction: Fumonisins are mycotoxins produced mainly by *Fusarium verticillioides* and *Fusarium proliferatum,* fungi commonly associated to maize. They are a group of structurally related compounds, fumonisin B1, B2 and B3 being the most found in maize. The consumption of food highly contaminated with fumonisins has been correlated with the development of esophagus and liver cancer, neural tube defects and cardiovascular diseases. Food thermal processing could lead to the formation of bound fumonisins - fumonisins bound through the tricarboxylic acids (TCA) side chains to starch or proteins, which are not detected by the usual analytical methods and can underestimate the levels of fumonisin in food. Hydrolysis under alkaline conditions, such as nixtamalization process (production of torillas), can break the link between TCA and the fumonisin backbone, releasing the hydrolyzed forms (HFB1, HFB2 and HFB3). Furthermore, bound fumonisins could also be hydrolysed in the gastrointestinal tract.
Objective: The objective of this work was to evaluate the hydrolysis conditions to enable the analysis of bound fumonisins in maize samples.
Methods: 50 µg of fumonisin standards (FB1, FB2, FB3) were evaporated to dryness, redissolved in KOH (2 M) and allowed to react in a thermal bath. To define the time required to fully hydrolyze fumonisins standards, two different procedures were compared: 60°C/ 30 min and 60°C/180 min. After hydrolysis, both mixtures were extracted with acetonitrile, the organic phase was pooled, evaporated under N_2, and redissolved in acetonitrile: water (1:1). In order to check out the yield of the reaction, a method of analysis of the parental compounds (FB1, FB2, FB3), fully hydrolyzed (HFB1, HFB2, HFB3) and partially hydrolyzed (PHFB1, PHFB2, PHFB3) was developed using LC-MS/MS (4000 QTRAP – ABSciex).
Results: Analysis of the fumonisin standards submitted to the milder hydrolysis conditions (60°C/ 30 min) showed that the binomial time-temperature was not sufficient to break all fumonisin present, since the parental compounds were still found (no partially hydrolyzed fumonisins). Analysis of the standard solutions that have undergone the more severe hydrolysis procedure (60°C/180 min) did not show any response for the parental compound or for the partially hydrolyzed fumonisins, demonstrating that the hydrolysis was complete.
Conclusion: Complete hydrolysis of fumonisins were achieved when at 60°C/180 min. This procedure will be used to release the bound fumonisin present in food thermally processed to enable the determination of the total fumonisin content.
Acknowledgments: CNPq, CAPES e FAP-DF.

FT 16- MERCURY MONITORING IN FISH SOLD IN MINAS GERAIS

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Introduction: The Ezequiel Dias Foundation participates of the program for monitoring the quality of food sold in the State of Minas Gerais in the conduct of the tests, in supervisory control, of samples collected by the State Health Surveillance. One of the parameters used for monitoring was the mercury in fish, which is provided in the RDC n° 42, of August 29, 2013, and indicates a maximum content of 1.00mg/kg for predator fish and 0.50mg/kg for non-predator fish. Between the years of 2013 and 2014 the Health Surveillance collected 29 samples of fish, being 14 units of frozen fish and 15 units of canned fish, which were sent for the execution of the test.
Objective: Evaluate the fish samples quality, both frozen and canned, on the content of mercury.
Materials and Methods: The experiments were performed by atomic absorption spectrometry, by thermal atomization, using the mercury analyzer model MA-3000 Nippon Instruments Corporation, coupled to a balance Mettler Toledo, model MS204S, all managed by software. The samples were homogenized, weighed in ceramic sample boats, in triplicate, always using a certified sample for the analytical control and were placed in the equipment automatic sampler to start the tests. The method limit of quantitation (LQ) was determined to be 0.015mg/Kg.
Results and Discussion: Between 2013 and 2014, it was analyzed 29 fish samples, both canned and frozen. Among canned samples, there were 14 Tunas and 1 Skipjack Tuna, and all showed mercury content above the LQ, in concentrations ranging between 0.03 and 0.38mg/kg, and thus below the maximum provided in the legislation for that kind of fish (0.50mg/kg). Among the 14 frozen fish samples, 7 presented mercury content below the LQ and in the other 7 it was quantified, with values ranging from 0.018 to 1.87mg/kg. Only one sample showed unsatisfactory result, with content above the established by legislation for predator fish (1.00mg/kg).
Conclusion: Considering the results, it can be stated that the samples sold in the State of Minas Gerais have good quality regarding the content of mercury, since only one sample exceeded the limit recommended by the current legislation. The sample that showed the highest mercury content was Requiem Shark that, by its predatory characteristic, justifies the accumulation of mercury in its tissues. The fact that the majority of samples submitted a quantifiable mercury content demonstrates the importance of the continuity of the monitoring program in this type of food.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EXPERIMENTAL TOXICOLOGY

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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EX 01- EFFECTS OF MATERNAL EXPOSURE TO ELECTROMAGNETIC RADIATION EMITTED BY MOBILE PHONE DEVICE: MATERNAL TOXICOLOGY AND EMBRYONIC DEVELOPMENT

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Introduction: The effect of electromagnetic radiation (EMR) emitted by mobile phones on biological systems has been extensively discussed by public and scientific community. It has been questioned if the EMR from mobile phones is capable of affecting biological systems. Any embryotoxicity of EMR in pregnant mothers would naturally be of great concern and experimental investigations of this possibility have been recommended by WHO. **Objective:** To evaluate if the EMR from mobile phones induces maternal toxicity in pregnant rats during the early stage of pregnancy and the effect of this kind of radiation on embryonic development. **Methods:** Approved by the CEEA/UFJF (Protocol 52/2012). Wistar rats in the 10th day after intercourse were randomized into two groups: exposed to radiation (n= 10) and not exposed (n= 10). The exposure occurred from the 1st to the 15th day of pregnancy through mobile phone calls of 25 seconds every 2 minutes for 12 h in the active period of the rats. Euthanasia occurred on the 15th day of pregnancy by exsanguination under anesthesia. It was assessed maternal and fetal variables. Maternal: presence of clinical signs of toxicity; food intake; weight gain; organ weights (liver, kidneys, adrenals, spleen, and ovaries); number of corpora lutea; proportion of implants, pre and post-implantation losses per group and average weight of placentas/litter; fetal variables: average weight of fetuses/litter and external fetal malformations. In serum were measured 17 β- estradiol, progesterone and hydrogen peroxide. The ovarian follicular population was counted and the diameters of tertiary follicles were measured by histological analysis. Statistics: Student's T test ($p \leq 0.05$). **Results and discussion:** There were no clinical signs of maternal toxicity. Food intake, body weight gain and the weight of maternal organs were similar between the groups. Number of corpora lutea showed a significant reduction of 6.45% in the exposed group. Proportion of implants, pre and post-implantation losses were not significantly different. Fetal weight/litter and placentas weight/litter were similar between the groups. External fetal malformations were not observed. Progesterone was similar in both groups, while 17 β-estradiol increased by 8.6% in the exposed group and hydrogen peroxide showed a 1.6-fold increase in the exposed group compared to the control group. Follicular population was similar in both groups, but the diameter of the tertiary follicles decreased by 14.77% in the exposed group. **Conclusion:** Pregnant rats exposed to EMR from mobile phones during the initial stage of pregnancy exhibited number of corpora lutea and tertiary follicle diameters decreased, hormonal changes and peroxide increased, which may interfere in the embryonic development.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 02- EFFECTS OF MATERNAL EXPOSURE TO ELECTROMAGNETIC RADIATION EMITTED BY MOBILE PHONE DEVICE: ADRENAL FUNCTION OF ADULT MALE RAT OFFSPRING

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Introduction: Due to the increased use of wireless and mobile phone communication devices in recent years, there is concern about the possible harmful effects of prolonged exposure to radio frequency radiation. Negative effects have been demonstrated on the nervous and reproductive systems, but little is known about the programming effect of radiation emitted by cell phones. Maternal and environmental factors during the perinatal period permanently affect the physiology and metabolism of offspring, increasing the risk of developing diseases in adulthood. The hypothalamic-pituitary-adrenal axis (HPA) plays a central role in responding to environmental variations throughout life. In this context, changes in catecholamine profile, which is an important mediator of stress reaction and behavioral variations, may indicate that the HPA axis was affected by the radiation of cell phones. **Objective:** Determine the effects of radiation emitted by mobile phones during pregnancy on programming of the adrenal gland and behavior in adult rat offspring. **Methods:** Approved by the CEEA/UFJF (Protocol 98/2012). Pregnant Wistar rats were randomly divided into two groups: not exposed (n = 10) and exposed to radiation (n = 10). The exposure occurred from the 1st to the 20th day of pregnancy through mobile phone calls of 25 seconds every 2 minutes for 12 h in the active period of the rats. The open field and inhibitory avoidance behavioral tests were carried out in male offspring at 90 days of age. At the end of the tests the animals were euthanized and the adrenal glands were immediately removed to quantify the total content and basal secretion of catecholamines. The fasting serum glucose was also measured. Statistics: Student's t test ($p \leq 0.05$). **Results and discussion:** No significant differences were observed in the behavioral tests, indicating the absence of effect on anxiety profile and long-term memory of animals programmed by radiation emitted from mobile phone. A significant increase (+41.5%) was observed in the total content of catecholamines of the exposed group, while there was a significant decrease (-50,7%) in basal secretion of the exposed animals. The serum glucose was lower (-15.4%) in exposed group than in control group, which may be due to the decreased catecholamines secretion. **Conclusion:** The animals programmed by radiation from mobile phones presented metabolic changes related to catecholamine secretion and glucose serum levels, but no changes in behavioral tests.

Acknowledgement: FAPEMIG, CAPES, UFJF and CNPq

EX 03- MULTIVARIATE STATISTICAL METHODS IN THE INVESTIGATION OF THE ANXIOLYTICS EFFECTS OF KETAMINE

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Introduction: Ketamine (KT) is an anesthetic agent with anxiolytic properties[1]. Zebrafish (*Danio rerio*) has become an interesting animal model for pharmacological studies due to their physiological and genetics similarities with mammals, to its robust behavioral responses and their sensitivity to psychoactive substances. The validation and interpretation of results of behavioral assays requires a suitable statistical approach and the use of multivariate statistical methods has been little explored, especially in zebrafish behavioral models. **Objective:** Validation of a method for analyzing typical anxiety behavior induced by KT in adult zebrafish using PCA, HCA and SIMCA. **Materials and Methods:** A 115 adult zebrafish were placed in 1000 mL tanks and exposed to KT for 20 minutes. The selected concentrations of KT were 5 (n = 20), 20 (n = 17), 40 (n = 32) and 60 $mg.L^{-1}$ (n = 32)[1]. The control group (n = 14) was exposed to the drug-free water. The behavioral effects were assessed through the Light-Dark Box Test. Zebrafish was placed in the dark side of the tank, faced to the wall and its behavior was recorded for 5 min. The behavioral parameters evaluated manually were the number of midline crossings, the time spent in the light area, the latency to the first access to the light area and the average entry duration in light area. In statistical analysis, variables were the behavioral parameters with numerical values autoscaled. **Results and Discussion:** PCA showed that 2 principal components accounted for 88.74% of all the system information. Behavioral similarity is observed among the control and the group exposed to 5 mg/L and among the groups exposed to 40 and 60 mg/L. Zebrafish exposed to 20 mg/L showed an intermediate behavioral profile. HCA analysis indicated the division of the samples in 5 groups, which is coherent with the number of experimental groups. SIMCA and HCA indicated a logical dose-dependent evolution of the samples in each class. The control generated a more dispersed behavioral profile. Treatment groups presented a more homogeneous behavioral profile, suggesting a KT induced-standardization of behavior. The time spent in light area and number of crossings were the best parameters to model the Classes and the number of crossings and the latency were the better endpoints to discriminate two groups. KT reduced the latency to light area and increased the time spent in the light area, which corresponds to a reduction of anxiety -like effects. KT also increased the number of midline crossings, which corresponds to increased locomotor activity. **Conclusions:** Multivariate statistics provide a more detailed and comprehensive approach to behavioral parameters analyses and can be applied to other behavioral tests in the analysis of other drugs.

Reference: [1] R. Riehl et al., *Neurotoxicol Teratol*. **33**, 6 (2011).

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 04- LOW TOXICITY OF AN EXTRACT RICH IN FLAVONOL-O-3-GLYCOSYLATED FROM LEAVES OF *Turnera subulata* WITH ANTICOAGULANT ACTIVITY

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Introduction: Folk medicine studies have been attracting scientific attention. An increasing number of research groups engaged in identification and chemical characterization of new active principles to elucidate the pharmacological action of these substances. The *Turnera subulata* (*T. subulata*) popularly known as "chanana", a small shrub, characterized as a weed, belonging to Passifloraceae family. The *Turnera* genus includes about 135 species widely distributed in tropical and subtropical regions of the Americas and Africa. Its popular use as herbal medicine with anti-inflammatory, expectorant and antidiabetic activities has been described in the literature.
Objective: The objective was to chemically characterize the extracts obtained from the leaves of *T. subulata* and evaluate its effect on blood clotting and toxicity *in vivo*.
Material and Methods: After collection, the leaves were stabilized in an air circulating oven at 40 °C for 24 hours and milled. The extraction was carried out with 50% ethanol, and fractionated with ethyl acetate. The extracts were, then, filtered and lyophilized. The chemical composition was determined by High-Performance Liquid Chromatography (HPLC). Activated Partial Thromboplastin Time (APTT) was used to assess the anticoagulant potential of extracts and toxicological tests were perfommed in Wistar rats (200-300g) and carried out according to Organization for Economic Co-operation and Development (OECD). This study was approved by the Ethics Committee on Animal Use (UFRN) under number 029/2011. Hematological and biochemical parameters of hepactic and kidney funcition, as well as, markers of glucose and lipids metabolism were determined. Drinking water, feed and the animal weight, were also assessed.
Results and Discussion: This study showed that extracts from *Turnera subulata* leaves have as major compound a flavonol-O-3-glycosylated like rutin (bioflavonoid). These compounds have the ability to completely inhibit the intrinsic coagulation pathway (APTT assay) in a concentration of 50µg/ml. The studied extracts also did not cause toxicity in the concentration used (2000mg/kg). No significant difference was found between control and treated group, for the hematological and biochemical parameters, as well as, for animal weight, water and feed consumption.
Conclusion: Based on these data, the studied extract has a satisfactory anticoagulant capability with low toxicity in vivo.

EX 05- (ANTI)GENOTOXIC ACTIVITY OF AQUEOUS EXTRACTS FROM THE PERICARP OF *Passiflora edulis* var. *flavicarpa* DEGENER AND OF *Passiflora edulis* var. *edulis* BY MICRONUCLEUS ASSAY IN RATS BONE MARROW

ARRUDA A.C.C.[1], ASSIS T.K.[2], DUARTE G.M.[2], BORGES V.S.[2], FÉLIX M.B.[2], MAIA D.H.S.C.[2], SOUSA A.P.S.[2], SANTOS P.J.S.S.[2], ZUCOLLOTO S.[2], SCHWARZ A.[3]

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Introduction: Species of Passiflora are used in folk medicine as sedatives and tranquilizers, but also as anti-inflammatory and diuretics. The pericarp of *Passiflora edulis* var. *flavicarpa* Degener and *Passiflora edulis* var. *edulis* are now being investigated for medicine purposes. There are no reports about it toxicity. **Objectives:** Assess the presence of micronuclei in bone marrow of rats treated with an aqueous extract of the pericarp of both species. **Methods:** 32 adult male Wistar rats were divided into four groups (n=8/group). The experimental groups received the aqueous extract (300 mg/kg) by gavage during 30 days, and the control groups received only the vehicle. On day 29 of treatment half of the animals of each group received an unique dose of cyclophosphamide (50mg/kg; i.p.) and the other half received 0,5 mL of sterile saline 0,9% (i.p.). At day 30 the animals were euthanized. The femur was exteriorized and bone marrow collected. Centrifuge tubes containing the bone marrow cells were centrifuged for 5 minutes at 1000 rpm, discarding the supernatant. Two smears were performed per animal. May-Grunwald-Giemsa stain (Merck) was used, modified by Rosenfeld. Staining allowed for differentiation between polychromatic (PCE) and normochromatic erythrocytes (NCE). (Anti)genotoxic activity was assessed using the frequency of micronucleated polychromatic erythrocytes (MNPCE) in 1000 polychromatic erythrocytes, for each animal. The polychromatic erythrocyte count in 200 total erythrocytes (PCE + NCE) by the PCE/NCE ratio, in order to confirm the presence of cytotoxicity, was also determined. The procedures with animals were approved by CEUA-UFRN (nº: 032/2012). **Results:** Statistical analysis (mean ± SEM) revealed absence of changes in the frequency of MNPCE [*Passiflora edulis* var. *flavicarpa* (negative control: 3.26±0.42; positive control: 11.72±1.02; negative experimental: 4.02±0.13; positive experimental: 10.47±0.87) and *Passiflora edulis* var. *edulis* (negative control: 2.84 ± 0.45; positive control: 9.32 ± 0.83; negative experimental: 2.62±0.48; positive experimental: 9.23±0.96)] or cytotoxicity [*Passiflora edulis* var. *flavicarpa* (negative control: 0.37±0.08; positive control: 0.23±0.05; negative experimental: 0.37±0.07; positive experimental: 0.23±0.02) and *Passiflora edulis* var. *edulis* (negative control: 0.32±0.08; positive control: 0.13±0.06; negative experimental: 0.28±0.12; positive experimental: 0.15±0.03)]. **Conclusion:** The aqueous extracts were not able to promote citotoxicity nor (anti)genotoxicity in rats at the doses used and conditions adopted in this study, since the clastogenicity of the cyclophosphamide were not influenced by the aqueous extract. **Acknowledgments:** Capes.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 06- DETERMINATION OF MALONDIALDEHYDE BY HPLC-PDA AS A BIOMARKER FOR OXIDATIVE STRESS APPLICATION TO HEPATOPROTECTIVE EFFECT OF HYDRO ALCOHOLIC EXTRACT OF THE AGROINDUSTRIAL WASTE OF JABOTICABA (MYRCIARIA CAULIFLORA O. BERG) AND ETHANOLIC EXTRACT OF BREADFRUIT LEAVES (ARTOCARPUS ALTILIS (PARKINSON) FOSBERG, IN MICE.

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Introduction: The nonalcoholic fatty liver disease is the most common and emerging liver disease worldwide, and worsens by free radicals production. Studies on new sources of antioxidants are performed every year in an attempt to minimize the damage caused by free radicals. Plant species *Myrciaria cauliflora* (peels of fruits) and *Artocarpus altilis* (fruits and leaves) rich in phenolic compounds have a potential antioxidant. **Objectives:** To evaluate the hepatoprotective effect of hydro alcoholic extract of the agroindustrial waste of jaboticaba (HEJB (*M. cauliflora*) and ethanolic extract of breadfruit leaves (EEBL) (*A. altilis*) in experimental models of hepatotoxicity carbon tetrachloride (CCl_4). **Methodology:** Were used Swiss mice, males, divided in 8 groups. Five control groups (I: no treatment; II: olive oil, 10 mL/kg, i.p.; III: Propylene glycol 50%, 10 ml/kg, p.o.; IV: 0.3% CCl_4 in olive oil 10 mL/kg, i.p.; V: Silymarin 200 mg/kg, p.o., positive control) and four treated groups, p.o. (VI: HEJB 250 mg/kg, VII: HEJB 500 mg/kg, VIII: EEBL: 250 mg/kg). Except groups I and II, all others were treated with 0.3% CCl_4 in olive oil on the 7th day of treatment, 2 hours after p.o. administration. At the end of the treatments, the animals were euthanized, had their blood drawn for conducting biochemical and hematological tests and had macroscopic necropsy. The potential hepatoprotective and antioxidant activity of plant extracts were observed through the hepatic enzyme (ALT), (AST), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) and the dosage of malondialdehyde (MDA) - which required validation analytical HPLC-PDA. **Results and discussion:** The chromatographic method offered satisfactory linearity with r>0,99 (0,5 a 40nmol/mL); limit of detection of 0,25nmol/mL and limit of quantification of 0,5 nmol/mL; the tests of de selectivity, precision, accuracy, stabilities, matrix and residual effect showed variations lower that 15%. For analysis of MDA in the liver, the IV group showed higher concentrations than all groups ($p<0.0001$), showing the intoxication of animals, as well as protection against free radicals, on groups treated with the extracts. The AST/ALT ratio showed the injury caused by CCl_4 in comparison to control groups. There was no significant difference for the CAT and GPx tests. For GR activity was observed a significant difference between the V and VII groups ($p<0.05$) compared to group IV. **Conclusion:** The bioanalytical method proved to be suitable for the dosage of MDA in plasma and liver. There was a decrease of MDA in liver tissue, for the two extracts, as well as decreased levels of AST/ALT, GR to HEJB showed that agroindustrial waste of jaboticaba fruit peel showed antioxidant activity in vivo.

EX 07- ENVIRONMENTAL TOBACCO SMOKE IN THE EARLY POSTNATAL PERIOD DISTURBS MYELIN-SPECIFIC PROTEIN

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Introduction: Several substances can induce neurotoxicity during brain development. Studies in rodents have shown that these effects are more evidents when the exposure occurs during the first two postnatal weeks, period characterized by the rapid head growth and by processes like myelination. Previous study from our group showed that environmental tobacco smoke (ETS) exposure during postnatal early brain development induce impairment in cognitive functions that may be associated with injury in synaptic transmission. However, little is known about the effects of ETS in myelination. **Objective:** The aim of this study was to evaluate the effects of tobacco smoke in the axonal myelination in optic nerve and in the protein expression at different stages of myelination: Olig-1, a marker early stage and myelin basic protein (MBP), a marker of late stage. **Methods:** BALB/c mice were exposed to a mixture of central and lateral tobacco smoke reference 3R4F from the 3rd day of life (P3) to 14th (P14) during 2h/day. Animals (n=6) were euthanized at P15 (childhood), P35 (adolescence) and P65 (adulthood). To evaluate protein expression of Olig-1 and MBP were performed in cerebellum, brainstem, diencephalon and telencephalon by immunoblotting assay. The analysis of axonal myelination was performed by couting myelinated fibers in transmission electron microscopy. **Results and Discussion:** Mice exposed to ETS showed significant increase in MBP ($p<0.05$) and significant decrease in Olig-1 ($p<0.05$) in cerebellum and brainstem in childhood, while in adolescence showed significant decrease in MBP in cerebellum ($p<0.05$) and brainstem ($p<0.05$). In adults mice exposed to tobacco smoke in the early postnatal there was significant decrease MBP in cerebellum ($p<0.05$). Moreover, we observed significant decrease in percentage of myelinated fibers compared with control only in childhood ($p<0.05$). Our results suggest that exposure to tobacco smoke in the early postnatal period induce changes in myelin-specific protein in critical phase of expression. Decreases in myelin-specific proteins in brainstem are particularly relevant. The exposure to ETS is considered one of the major risk factors for SIDS. The mechanism of SIDS is still unknown, but requires immature cardiorespiratory control and impairment in sleep arousal, involving the brainstem. Moreover, ETS induced a significant decrease in the number of myelination fibers in childhood, but these impairment also seem to be reverted when the animal reach the adolescence and adulthood. **Conclusion:** The exposure to ETS in the early postnatal period interfere in the myelination and induces persistent changes in critical brain structures since we demonstrated changes in myelin-specific protein in infancy, adolescence and adulthood.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 08- EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE (ETS) IN THE EARLY POSTNATAL PERIOD: PREDISPOSITION TO ADDICTION TO DRUGS OF ABUSE IN ADOLESCENCE

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Introduction: Brain development represents a period of vulnerability and several substances can induce neurotoxicity in this phase. Previous study from our group[3] showed that ETS exposure during the early postnatal period induces impairment in cognitive functions that may be associated with injury in synaptic transmission. However, little is known about the effects of the early exposure to ETS in predisposition to addiction. **Objective:** The aim of this study is to evaluate whether exposure to ETS in the early postnatal period contributes to the development of addiction to drugs of abuse during adolescence. **Material and Methods:** *Swiss-Webster* male mice were exposed to a mixture of central and lateral tobacco smoke of reference cigarettes 3R4F from the 3rd (P3) to the 15th (P15) day of life, twice a day. During adolescence, the animals (n=6) were challenged with cocaine or ethanol *(i.p)* and the evaluation of cross-behavioral sensitization (P35) and conditioned place preference (P36-45) were evaluated. Furthermore, by western blotting (n=6), biochemical marchers of neuroadaptation in the striatum and the pre frontal cortex, like dopamine receptors (D1 and D2), Rac1, c-fos and FRAs were quantified. **Results and Discussion:** By time slot, conditioned place preference (CPP) indicates preference for the CS^+ (conditioned stimuli) in ETSETA group (range 0-5 minutes) and preference for CS^+ in ETSCOC group (range 20-25 minutes). Our results suggest that the significant decrease in preference to the CS^+ of animals in ETSSAL group compared to CTSAL may be due to the decrease in locomotors activity and impaired learning and memory, observed in our group studies.[3] During open field -testing, statistically significant was not observed for the group treated with ethanol. Decrease signaling Rac1 in nucleus accumbens increased the formation of dendritic spines.[1] Western Blotting assays showed this same decreased for Rac1 were observed in our results, however, in the pre frontal cortex for ETSSAL group. This suggests that plasticity mechanisms would act in the formation of new dendritic spines due to the long-term effects of exposure to tobacco smoke. In the striatum, c-fos gene expression is known to stimulate D1 receptors in the presence of the drug.[2] Our results suggest that in the striatum there were a decrease in D1 receptor for PTASAL group compared to CTSAL group, as for the c-fos there were a decrease in PTACOC group compared to CTCOC group. This decrease of c-fos founded in our results was consistent, because we could consider the CPP test day a period of acute withdrawal. **Conclusion:** On the basis of the results recorded exposure to ETS can lead to increased vulnerability to the development of addiction to drugs of abuse.

1. M. Ghosh *et al*. *Science*. **304**, 743 (2004).
2. M. Morelli *et al. J Pharmacol Exp Ther*. **260**, 402 (1992).
3. L.H.L Torres-Pacheco. PhD Thesis (2013)

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 09- BISPHENOL A: NEUROTOXIC EFFECTS IN PRIMARY CULTURE HIPPOCAMPAL

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Introduction: Bisphenol A (BPA) is used in the manufacture of polycarbonate plastics. Prenatal exposure to BPA may lead to development problems in the reproductive system and increase the risk of cancers. Little is known about the effects of BPA in neuronal cells.
Objectives: This work aims to study the neurotoxicity of BPA in primary culture of hippocampus.
Material and Methods: Cultures of primary hippocampal neurons were exposed to BPA at concentrations of 50, 100, 150, 200 and 250 µM for 6, 12, 24 and 48 hours. Cell viability was assessed by MTT method (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide); for the assessment of cytotoxicity was used CytoTox-ONE ™ Homogeneous Membrane Integrity Assay Kit - Promega for the evaluation of release of the enzyme lactate dehydrogenase (LDH). Cells (2x106 cells/well in 6-well plates) were treated with 200 and 250 µM of BPA for 3, 6 and 9 hours and the proteins (Bax, Bcl-2, caspase-3, caspase-8, caspase-9 and cytochrome c) were analyzed by Western Blotting assay.
Results and Discussion: Cells exposed to concentrations of 200 and 250 µM of BPA of 12, 24 and 48 hours, showed a significant decrease in cell viability compared to control. For the cytotoxicity assay, release of LDH into the culture medium was not statistically significant for any of the exposure concentrations and periods of incubation. This suggested that BPA leads to neuronal death in the concentration of 200 and 250 µM in periods of exposure (12, 24 and 48 hours) and observed that there was no release of LDH into the culture medium, indicating no disruption of the plasma membrane, suggesting that BPA does not cause death by necrosis. Western Blotting analysis was observed a decrease of Bcl-2 in the concentration of 200 µM in 3 and 6 hours exposure and also to concentration of 250 µM in 6 hours exposure. An increase in the quantification of Bax/Bcl-2 to the concentration of 200 µM in the exposure of 6 hours compared to controls was observed. This indicates that the pro-apoptotic effect may be more pronounced for the concentration of 200 µM in a 6 hour exposure. We also observed the presence of protease caspase-8 and caspase-3 in their pro-forms and in their cleaved forms, for caspase-9 has been observed only a cleaved form, indicating that BPA can induce cell death both the intrinsic and extrinsic pathway.
Conclusion: Based on our data, was possible to observed that BPA decrease cell viability and not cause cell death by necrosis. BPA possibly cause cell death by both the intrinsic and extrinsic pathway of apoptosis.

EX 10- PERINATAL EXPOSURE TO TYPE I OR TYPE II PYRETHROIDS PROVOKE BEHAVIORAL *IMPRINTING* DURING OFFSPRINGS DEVELOPMENT.

GODINHO, A.F.[1]; YASSUDA M.M.[1]; ANSELMO, F.[1]; FARIA, C.A.[1]; KAWASHIMA, J.D.[1]; HORTA, D.F.[1]; SILVA, D.A.F.[1]; DE FRAIA, D.[1]; SOUZA, A.C.O.[1]; CARVALHO, C.C.[1]

[1]Laboratório de Pesquisas Neurocomportamentais (LAPEN), Centro de Assistência Toxicológica (CEATOX), Instituto de Biociências de Botucatu (IBB), Universidade Estadual Paulista (UNESP), Botucatu, São Paulo.

Introduction: Pyrethroids are a group of insecticides know by provoke nervous paralysis for drawing out the opening sodium channels of the cellular membrane delaying the repolarization and by increase of nervous excitability due to inhibition of the chlorine flow regulated by γ-aminobutyric acid (GABA). **Objective:** Evaluate physical and sensory motor development following birth and behaviors like anxiety in young (22 days-P22) and adult (75 days-P75) pups of mothers exposed during gestation and lactation, to allethrin (All-type I) and cypermethrin (Cyp-type II) pyrethroids. **Material and Methods:** Pregnant Wistar rats (N=15) received orally (gavage), throughout pregnancy and lactation: corn oil as control (Ct), d-allethrin (43mg/kg), and cypermethrin (12,5mg/kg) treatments. At birth, litters of acceptable size were sexed, weighed, adjusted to six male pups per mother, and maintained with it until the 21 day age. During the first days after birth pups were evaluated for parameters of somatic and sensory motor development. Pups at ages of 22 and 75 days were tested for social interaction(SI) using the Open Field (OF) and anxiety behavior using the Elevated Plus Maze (EPM) apparatus. Pyrethroids dosage (HPLC) was made in blood of mothers (21 day gestation) and pups (at P1, P22, and P75). **Results and Discussion:** Was detected presence of All and Cyp in blood of mothers, P1, and P22, but not in P75 pups. Was observed significant decreased in time for incisor eruption, first appearing of hair, and ear unfold, for both All and Cyp. Eye opening and testes descent were unchanged by All or Cyp. The time for disappearance of palmar grasp reflex increased (All and Cyp); the time for appearance of postural and negative geotaxis were increased significantly (All and Cyp). The acoustic startle responses decreased significantly (All and Cyp). In OF the SI, which is a index of anxiety behavior, was decreased significantly by both All and Cyp at P22 and P75. In EPM, All and Cyp perinatal exposition provoked a significant decrease in open arms entries and in time spent in open arms, and a significant increase in closed arms entries and in time spent in closed arms, in P22 and P75 pups, demonstrating an anxiogenic effect by both type I and type II pyrethroids studied. Alterations in somatic and sensory motor development of pups exposed and increased anxiety behavior during development that persisted into adult age same in absence of insecticides, suggest a effect of *imprinting* by pyrethroids action. It is important to point out that the toxic responses here observed were not influenced by the type of pyrethroid, if I or II. **Conclusion:** The present results demonstrate that pyrethroids developmental neurotoxicity occurs in cases of mothers perinatally exposed.
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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 11- PERINATAL EXPOSURE TO TYPE I OR TYPE II PYRETHROIDS PROVOKE BEHAVIORAL *IMPRINTING* DURING OFFSPRINGS DEVELOPMENT.

GODINHO, A.F.[1]; YASSUDA M.M.[1]; ANSELMO, F.[1]; FARIA, C.A.[1]; KAWASHIMA, J.D.[1]; HORTA, D.F.[1]; SILVA, D.A.F.[1]; DE FRAIA, D.[1]; SOUZA, A.C.O.[1]; CARVALHO, C.C.[1]

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Introduction: Pyrethroids are a group of insecticides know by provoke nervous paralysis for drawing out the opening sodium channels of the cellular membrane delaying the repolarization and by increase of nervous excitability due to inhibition of the chlorine flow regulated by γ-aminobutyric acid (GABA). **Objective:** Evaluate physical and sensory motor development following birth and behaviors like anxiety in young (22 days-P22) and adult (75 days-P75) pups of mothers exposed during gestation and lactation, to allethrin (All-type I) and cypermethrin (Cyp-type II) pyrethroids. **Material and Methods:** Pregnant Wistar rats (N=15) received orally (gavage), throughout pregnancy and lactation: corn oil as control (Ct), d-allethrin (43mg/kg), and cypermethrin (12,5mg/kg) treatments. At birth, litters of acceptable size were sexed, weighed, adjusted to six male pups per mother, and maintained with it until the 21 day age. During the first days after birth pups were evaluated for parameters of somatic and sensory motor development. Pups at ages of 22 and 75 days were tested for social interaction(SI) using the Open Field (OF) and anxiety behavior using the Elevated Plus Maze (EPM) apparatus. Pyrethroids dosage (HPLC) was made in blood of mothers (21 day gestation) and pups (at P1, P22, and P75). **Results and Discussion:** Was detected presence of All and Cyp in blood of mothers, P1, and P22, but not in P75 pups. Was observed significant decreased in time for incisor eruption, first appearing of hair, and ear unfold, for both All and Cyp. Eye opening and testes descent were unchanged by All or Cyp. The time for disappearance of palmar grasp reflex increased (All and Cyp); the time for appearance of postural and negative geotaxis were increased significantly (All and Cyp). The acoustic startle responses decreased significantly (All and Cyp). In OF the SI, which is a index of anxiety behavior, was decreased significantly by both All and Cyp at P22 and P75. In EPM, All and Cyp perinatal exposition provoked a significant decrease in open arms entries and in time spent in open arms, and a significant increase in closed arms entries and in time spent in closed arms, in P22 and P75 pups, demonstrating an anxiogenic effect by both type I and type II pyrethroids studied. Alterations in somatic and sensory motor development of pups exposed and increased anxiety behavior during development that persisted into adult age same in absence of insecticides, suggest a effect of *imprinting* by pyrethroids action. It is important to point out that the toxic responses here observed were not influenced by the type of pyrethroid, if I or II. **Conclusion:** The present results demonstrate that pyrethroids developmental neurotoxicity occurs in cases of mothers perinatally exposed.

The present work was supported by CEATOX.

EX 12- CELLULAR EFFECTS OF THIRDHAND TOBACCO SMOKE

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Introduction: Many toxic compounds remain in the environment after the cigarette is extinguished and accumulate in the air or on surfaces. This exposure is termed Thirdhand Smoke (THS) and its risks are poorly known. **Aim:** To evaluate the effects of THS from smoker's home on the cellular development using three different types of in vitro tests.
Methods: Cellulose papers (25cm^2) were disposed in the furnishing surface in the room with no direct exposure to sunlight from one smoker's home and one nonsmoker's home. The collecting material remained one, three and seven days in the house. An area of equivalent size was cleaned with cotton wipe wetted with 1 mL of water. Samples were placed in centrifuge tubes and immersed in Dulbecco's Modified Eagle's medium (DMEM) or methanol at a ratio of 50 mg material to 2 mL solution. The flasks were stirred to separate the supernatant from suspended particles. Free smoke paper and cotton wipe were processed in the same way and used as reference. Samples in methanol were used to measure nicotine by GC/MS. For exposure to THS, A549 cells were seeded at a rate of 2 x 10^4 cells/well in a 96-well and 1 x 10^5 cells/well in a 24-well plate polystyrene microplate and incubated until subconfluency. Then culture medium was replaced with DMEM + THS. The cells were exposed for 24 hours to THS samples to assess cellular functions through of tetrazolium dye reduction (MTT), Neutral Red reuptake (NR) and Trypan blue exclusion assays.
Results and discussion: Smoker home showed to be a potential reservoir of THS pollutants. Concentration of nicotine in this place ranged from 53.5 to 131.5 ng/cm^2. Nicotine was below of the quantification limit in the nonsmoker's home. Cigarette smoke induces several biological effects and cytotoxicity is considered as a key step in the smoking-related pathological processes. Cellular proliferation was similar in all samples of THS exposure, however were observed changes in the cellular functions. Mitochondrial activity evaluated by MTT assay was lower in the paper samples of the smoker's home (20 to 30% below the reference) nevertheless the sample of cotton wipe resulted in increased activity (30% above the reference). The samples of seven days exposition showed lysosomal viability increased in both houses (about 10% above the reference).
Conclusion: Surface cotton wipe and cellulose paper sampling for nicotine is a simple collection method to assess THS contamination and distinguish between nonsmoking and smoking environments. Even showing no cytotoxic effect, the THS was able to cause changes in important functions for suitable cell survival. The results demonstrated MTT, NR and Trypan assays may be used in A549 cells to evaluate biological effects at the cellular level of the THS exposure.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 13- METHYLMERCURY (MeHg) SHORT-TERM EXPOSURE PROMOTES TOXIC EFFECTS IN A BROADLY FASHION IN MICE.

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INTRODUCTION: Most of studies that investigate methylmercury (MeHg) toxic effects in laboratory animals use long-term exposure protocols (several weeks or months) and focus in a single system disrupted by MeHg. However, it is possible that behavioral and/or metabolic changes observed in these studies are initiated after a short -term MeHg exposure (a few days) and not be restricted to a single body system. **OBJECTIVE:** Thus, the present study aimed to investigate whether a MeHg short-term exposure (14 days) would be able to promote toxic effects in a broadly fashion in mice**. MATERIAL AND METHODS:** Were used adult male Swiss mice (45 – 60 days old), exposed to MeHg solution (40 mg/L) in drink water during 14 days. On the 14th day, animals were submitted to rota rod test, beam walking test, pole test and hind limb clasping phenomenon in order to evaluate motor performance/coordination. After, were evaluated total cholesterol, HDL- and non-HDL cholesterol, urea and creatinine levels; liver weight, ALT and AST activity. Also, cerebellum was removed, weighed and processed for glutathione peroxidase and glutathione reductase assay as well as, BDNF, IL-6 and Hg levels determination. Experimental procedures were approved by CEUA/UFSC, protocol PP00745. **RESULTS:** MeHg exposure (40 mg/L) for 14 consecutive days induced locomotor deficit, observed in the rotarod test (19.40±4.84 s *vs.* 49.54±2.91 s), in the beam walking test (35.25±5.65 s *vs.* 17.30±1.93 s), in the hind limb clasping phenomenon (1.277±0.114 *vs.* 0.497±0.113) and in the pole test (latency to turn: 44.50±11.50 s *vs.* 11.30±4.32 s; time to descend: 89.67±15.20 s *vs.* 19.43±7.00 s). Also, MeHg exposure increased significantly total cholesterol (122.8±4.23 mg/dL *vs.* 104.3±2.56 mg/dL) and non-HDL cholesterol (47.76±5.85 mg/dL *vs.* 30.23±3.65 mg/dL) levels, serum AST (178.3±14.79 U/L vs. 121.1±16.80 U/L) and ALT (116.6±13.83 U/L vs. 70.16±8.164 U/L) enzymatic activities, increased liver weight (4.917±0.161 % vs. 4.132±0.182 %) and reduced significantly serum urea levels (41.46±1.470 mg/dL vs. 50.29±2.296 mg/dL). Furthermore, MeHg exposure reduced significantly cerebellum weight (22.33±1.89 % *vs.* 27.94±1.13 %), promoted Hg deposition in cerebellum (6.357±0.737 µg/g *vs.* 0.494±0.063 µg/g), increased cerebellar GR activity (28.41±0.77 nmol/min/mg *vs.* 23.08±1.41 nmol/min/mg), reduced cerebellar GPx activity (6.056±0.282 nmol/min/mg *vs.* 6.932±0.173 nmol/min/mg), increased cerebellar BDNF levels (0.826±0.076 pg/mg *vs.* 0.544±0.051 pg/mg) and reduced cerebellar IL-6 levels (0.269±0.017 pg/mg *vs.* 0.375±0.041 pg/mg). **CONCLUSIONS:** MeHg short-term exposure (14 days) promoted toxic effects in mice not restricted to a single body system, especially in the central nervous system, hepatic system and serum lipid levels.

EX 14– GENDER INFLUENCE ON MANGANESE INDUCED DEPRESSION-LIKE BEHAVIOR AND Mn AND Fe DEPOSITION IN DIFFERENT REGIONS OF CNS AND EXCRETORY ORGANS IN INTRAPERITONEALLY EXPOSED RATS

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Introduction: Manganese (Mn) is a metal that, moreover its essenciality, it can modulate the action of endogenous substances, as neurotransmitters. Many studies have been conducted assessing Mn neurotoxicity, however, Mn bioaccumulation in different brain tissues and behavior effects involving gender-specific studies are conflicted in the literature. **Objectives:** Compare Mn effects, after 30 days of intraperitoneal treatment, in male and female animals, submitted to forced swim and open field tests. After that were evaluated Mn and Fe tissue levels in CNS, liver and kidneys. **Materials and methods:** A total of male (n=40) and female (n=40) Wistar rats were divided into saline, Mn 1mg/kg, Mn 5mg/kg, Mn 10 mg/Kg, and imipramine (as forced swim control). Male and female rats were housed separately. After treatments, they were submitted to the forced swim training, for 15min. Post 24h, they were submitted to the test for 5min, followed by open field to evaluate a putative sedation or motor damage. Then, animals were euthanized by anestesia overdose followed by decapitation and the collected tissue were: striatum, hippocampus, brainstem, cortex, cerebellum, hepatic tissue, and renal tissue. Mn and Fe were determined by ICP-MS as Batista and colleagues (2009) proposed method. Statistical analyses used were ANOVA 2-Way, followed by Tukey test ($p<0.05$). **Results and discussions:** All male rats treated with Mn 10 mg/Kg died in the first week of treatment, which were not expected. For female Mn 10mg/Kg, five animals survived until the end of experiments. Regarding the behavior analysis, the results indicated a higher time of immobility for male Mn 1mg/kg compared to the others, while, in the open field, there were lower crossing and rearing frequencies, and also a higher latency to mobility for female Mn 5mg/kg, differing from the others. Brainstem was the tissue with more Mn depositon for females Mn 10 mg/Kg, while striatum and hippocampus were higher for both genders in lower doses. For male, hippocampus, striatum, cortex, and kidneys had significantly increased dose-dependent Mn accumulation compared to controls. For female, all the CNS dissected had significantly increased dose-dependent Mn accumulation compared to controls. However, on the hepatic tissue, there were no differences in Mn levels, only a trend in accumulation ($p = 0.083$) observed for female Mn 10 mg/Kg. In renal dissected, the accumulation was significant, suggesting the possibility of an increase in the production of metallothioneins and that these would eliminate Mn by the kidneys. The accumulation of Fe was not proportional to the Mn, which suggests that there are other ways of transporting Mn, which does not affect Fe status. **Conclusion:** This study considered that: (i) males were less tolerant of neurotoxic I.P. doses of Mn; (ii) the Mn presented significant accumulation on all CNS dissected studied, the most in the brainstem for higher dose, striatum and

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

hippocampus for lower Mn doses; (iii) there is a pro-depressive effect induced in the forced swim, reinforced by the hypoactivity in the open field, suggesting that Mn can contribute to the noradrenaline and serotonin reduction.

EX 15- PROLACTIN ACTS ACUTELY IN PROMOTING PIN LESIONS ON THE FEMALE PROSTATE OF MONGOLIAN GERBILS

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The female prostate was considered a vestigial organ by mid-1950, however, is now known, which is an active gland with relevant importance in reproductive functions, producing a fluid released during female ejaculation, which contributes for sperm nutrition and survival when it is already in female tract. Mongolian gerbil's (*Meriones unguiculatus*) female prostate is more voluminous and developed gland compared to other rodents, besides, it is found in a frequency of 80%. Prolactin is an important polypeptide hormone, secreted by pituitary gland lactotrophs, which presents, among others, functions involved with reproduction. The aim of this study was to evaluate the high doses effects of short and long term prolactin administration on the morphology of normal and castrated female gerbil prostate. For this, 90 days-old females were divided into the following groups ($n = 8$): *CO* (females under no surgical intervention, killed between 114 and 141 days-old, in proestrus phase), *CA* (females ovariectomized at 90 days-old and killed between 114 and 141 days-old), *PRL* (females submitted to sc administration of prolactin - 0.3 mg/kg - once a day, from 111 to 113 days-old (3 days-subgroup) and from 111 to 140 days-old (30 days-subgroup); *CAPRL* (females ovariectomized at 90 days-old and subjected to the anterior treatment). The prostatic complexes were processed for inclusion in historesin and the slides produced were stained with H&E. The evaluation of histological sections showed prostates from *PRL 3d* with several focus of pre-neoplastic lesions (prostatic intraepithelial neoplasia - PIN) with cribriform arrangement, accompanied by intense inflammatory process. The multiplicity of these lesions was significantly higher than *CO*. In *PRL 30d* the number of lesions was not statistically higher than *CO*, however, various portions of acinar epithelium eliminated inside the lumen were observed, which may indicate the gland's recovery from damage by desquamation of the affected epithelium. Between *CA* and *CAPRL 3d* was also observed a significant increase in PIN lesions, which demonstrates the proliferative character of prolactin even in suppression of endogenous sexual hormones. However, *CAPRL 30d* showed no significant differences in the lesions multiplicity compared to *CA*, so that the gland also presented desquamation process to recover itself from prolactin action. Prolactin shown to have acute action, promoting PIN lesions in female gerbil prostate with only 3 days of administration. This effect also occurred even in the suppression of endogenous sexual hormones, promoted by castration. As the prolactin administration time increases, the gland recovers itself from morphological lesions by epithelium desquamation.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 16- MECHANISMS OF NEUROTOXICITY OF CISPLATIN: EFFECTS ON NEURITOGENESIS AND NEUROPROTECTIVE EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE)

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Introduction: Cisplatin is a commonly used chemotherapeutic agent that have a broad spectrum of action against several solid tumors. Unfortunately, its clinical use is limited by several adverse effects, including peripheral sensory neuropathy (PSN). The mechanism underlying the neurotoxicity of cisplatin remains unclear. Although many compounds have demonstrated protective effect against the neurotoxicity induced by cisplatin, no effective treatment has been developed. Caffeic acid phenethyl ester (CAPE) is a propolis component with neuroprotective properties and its effect on the neuropathy induced by cisplatin has not been elucidated yet. **Objective:** This study aims to investigate the possible protective effect of CAPE against the neurotoxicity induced by cisplatin as well as the mechanisms by which cisplatin induces neurotoxicity. **Materials and methods:** PC12 cells were cultured in DMEM supplemented with 5% heat-inactivated fetal bovine serum, 10% heat-inactivated horse serum and 1% of antibiotic mixture (37ºC, 5% CO_2). Cytotoxicity was assessed by MTT assay. Neurite outgrowth was evaluated by using inverted phase contrast microscopy and the Image J software. The expression of neuronal proteins (GAP-43, synapsin I and synaptophysin) was measured by using Western blot technique. Statistical analysis: Mean ± SEM from three independent experiments were analyzed by using One Way ANOVA with Bonferroni multiple comparison test and GraphPad Prisma software (level of significance, $p < 0.05$). **Results and discussion:** Cisplatin (5 µM) did not cause significant cell death; however, it decreased the differentiation of NGF-stimulated PC12 cells as well as the expression of proteins associated with axonal growth (GAP-43) and synaptogenesis (synapsin I and synaptophysin). The addition of CAPE (10 µM) minimized the inhibition of cell differentiation induced by cisplatin and increased the expression of the three neuronal proteins analyzed, both in the presence and in the absence of cisplatin.
Conclusion: The neurotoxic effects of cisplatin were induced by non-lethal concentrations of the drug; therefore, down-regulation of neuronal proteins with resulting impairment of neurites growth and synaptic communication might be an early event in the neurotoxicity of cisplatin that is attenuated by CAPE. Further studies are necessary to delineate the pathways by which cisplatin decreases the expression of neuronal proteins and CAPE protects against it.

Acknowledgments: CNPq and CAPES.

EX 17- THE EFFECT OF INHIBITORS OF DNA METHYLATION ON MPP$^+$-INDUCED NEUROTOXICITY IN PC12 CELLS

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Introduction: Environment can significantly affect the risk and progression of Parkinson´s Disease (PD), a neurodegenerative disorder characterized by loss of dopaminergic neurons. DNA methylation is one of the major epigenetic mechanisms controlling gene transcription. It is a process catalyzed by DNA methyl transferases (DNMT) in which a methyl group is added to a cytosine before a guanine in CpG islands, a modification frequently associated with gene silencing. Studies suggest that DNA methylation is an important biological mechanism that alter gene expression and contribute to the development of neurodegenerative diseases, including PD. However, little is known about the effect of drugs that modify DNA methylation on the neurodegenerative processes associated with PD.
Objective: Evaluate the effect of DNMT inhibitors (5-aza-cD and RG108) on a dopaminergic neurodegeneration model (PC12 cells/MPP+) and the mechanisms involved.
Materials and Methods: PC-12 cells were cultured in DMEM medium supplemented with 10% horse serum, 5% fetal bovine serum (FBS) and 1% antibiotic mixture (PSN). Cell viability was evaluated by MTT assay. Briefly, cells were platted in 96-well plates; after 24h-incubation, 1mM MPP+ and/or DNMT inhibitors were added. For pretreatment assays, DNMT inhibitors were added 24h before MPP+ addition. After medium removal, cells were incubated with MTT for 3 hours and lysed with DMSO. Absorbance was determined at 570 nm. For neurite outgrowth assay cells were incubated in growth medium for 24h in 24-well, poly-L-lysine coated plates. Then, the medium was replaced by F12K supplemented with 1% of horse serum, 1% PSN, NGF 100 ng/mL and DNMT inhibitors. Quantitation was performed by using inverted phase contrast microscopy and the Image J open source software. For statistical analyses, one-way ANOVA with Dunnet´s multiple comparisons test (level of significance, $P < 0.05$) and GraphPad Prism software were used.
Results and Discussion: DNMT inhibitors did not affect the viability of PC12 cells when added alone or together with MPP+. However, pretreatment with DNMT inhibitors for 24h exacerbated the cell death induced by MPP+. Additionally, DNMT inhibitors alone decreased the neuritogenesis induced by NGF in PC12 cells. All together, these findings suggest that inhibition of DNA methylation impairs neuritogenesis making cells more vulnerable to MPP+ toxicity.
Conclusions: DNMT inhibitors exacerbate (pretreatment) or do not affect (simultaneous treatment) MPP+-induced cell death and decrease neurite growth in PC-12 cells. Additional experiments are necessary to understand the mechanisms involved in such effects.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 18- CYTOTOXIC AND MUTAGENIC EFFECTS OF THE COMBINED USE OF SACCHARIN AND CYCLAMATE

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Introduction: The use of saccharin and sodium cyclamate as food additives was always controversial due to its association with carcinogenesis, making countries such as USA and Japan prohibit their use as non-sugar sweeteners. Brazilian current legislation (ANVISA - Health Surveillance Agency) still allow their use despite the risks, and has proposed a maximum acceptable daily intake (ADI). Although they have been proved to be toxic, at this point, there is no study evaluating the combined use of these additives as sweeteners. **Objective:** The study aimed to evaluate the cytotoxic and mutagenic potential of the combined use of saccharin and sodium cyclamate sweeteners in both animal testing system (mice) and plant test system (*Allium Cepa*). **Materials and Methods:** Both test systems used were exposed for 7 consecutive days: Group 01 received only distilled water (negative control); Group 02 received half the maximum ADI recommended by Brazilian law - 10mg / 100g of saccharin and 20mg / 100g cyclamate; Group 03 received the maximum ADI - 15mg / 100g of saccharin and 40mg / 100g of cyclamate; Group 04 received twice the maximum ADI 30mg / 100g of saccharin and 80mg / 100g cyclamate; and Group 05 received cyclophosphamide 50mg / kg (Positive Control). Blood samples and *Allium cepa* meristems were collected 48, 72 and 168 hours after treatment initiation for assessing mutagenicity (blood samples) and cytotoxicity and mutagenicity (*Allium cepa*). **Results and Discussion:** In the plant test system, concentration 01 presented cytotoxicity in the period of 168hs ($p < 0.001$), and was mutagenic in the period of 72h ($p < 0.05$). Concentration 02 was cytotoxic in all three analyzed period (48hs, 72, and 168hs) and concentration 03 was cytotoxic in the period of 48hs and 72hs ($p < 0.01$) and mutagenic on days 72 and 168hs($p < 0.05$). In the animal test system, concentrations 02 and 03 were mutagenic in 168hs ($p < 0.05$). Uncontrolled use of these non-sugar sweeteners is known to be cytotoxic and mutagenic in human cells, and, in this regard, we have observed that their combined used can synergistically enhanced their harmful effect. **Conclusion:** Concentrations 01 and 02, which are allowed according to the Brazilian legislation, presented cytotoxic and mutagenic effects. Therefore, we strongly recommend the revision the maximum ADI by the current legislation, since these sweeteners are used for a large portion of the population and for long period of time.

EX 19– IN VITRO ASSESSMENT OF THE EFFECT OF EXTRACTS OF AMAZON HERBS ON THE ENZYMATIC ACTIVITY OF HUMAN P-GLYCOPROTEIN

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Introduction: Drug interaction is problem of great interest in clinical practice. This phenomenon may produce different pharmacological responses due to the interference of food, beverages, addictive drugs or medicines on the action of a therapeutic agent. The drug transport is one of the main factors that dictates pharmacokinetics and, therefore, is a common mechanism of drug interactions. P-glycoprotein is a well-known efflux pump and has been linked to several clinically important drug interactions. However, to the best of our knowledge, no investigation has been made evolving extracts from amazon herbs. **Objective:** Investigate the effect of extracts from amazon herbs on the enzymatic activity of human P-glycoprotein. **Materials and Methods:** Some extracts with potential pharmacological activities previously described were selected: *Eugenia punicifolia* (pedra-ume-caá), *Caesalpinia ferrea* (jucá), *Astrocaryum tucuma martius* (tucumã), *Maytenus guyanensis* (Chichuá), *Myrciaria dubia* (camu-Camu), *Hymenaea courbaril* (jatobá), *Byrsonima japurensis* (Murici), *Cissus sicyoides* (insulina vegetal) and *Endopleura uchi* (uxi). The extracts were diluted to the concentration of 50 ug/mL. The processed samples were evaluated for flavonoids and total phenols content and for enzymatic activity using the luminescence-based "P-gP-Glo™ Screening Systems", which employs human recombinant P-glycoprotein. All samples were assayed in triplicates and statistical analyses were performed utilizing GraphPad Prism 6. **Results and discussion**: Jucá sample presented the higher contents of flavonoids and total phenols (42.0 and 6.7%, respectively). Only chichuá sample apparently estimulated P-glycoprotein. On the other hand, the other samples did not interact with this protein in the conditions used. **Conclusions:** According to the screening results obtained, chichuá sample may have the potential to estimulate P-glycoprotein in vitro. However, further studies should be done to confirm these findings and to clarify the mechanisms involved in this phenomenon.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 20- INVESTIGATION OF THE TERATOGENIC POTENTIAL OF *Pimenta pseudocaryophyllus* (GOMES) L.R. LANDRUM, (E)-METHYL ISOEUGENOL CHEMOTYPE, IN WISTAR RATS

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Introduction: Traditionally used medicinal herbs have been studied with the purpose of developing herbal medicines for primary care in the Unified Health System (SUS) in Brazil. In this way, a promising species named *Pimenta pseudocaryophyllus* (Myrtaceae), used in Brazilian folk medicine for several purposes, has been studied in the last decade, due to its wide pharmacological potential, including antibiotic, anxiolytic, hypotensive, antidepressant, photoprotective, antioxidant, antinociceptive and anti-inflamatory properties. However, there is a lack of knowledge regarding its toxicological profile, especially during pregnancy. **Objective:** This study aimed at investigating the toxicological effects of the dry leaf extract of *P. pseudocaryophyllus*, (E)-methyl isoeugenol chemotype (EPPm) in the external and skeletal development of Wistar rats offsprings. **Material and Methods:** Forty-two dams were divided in four groups which received EPPm at the doses of 0, 2000, 2500 or 3000 mg/kg/day. The extract was orally administered from gestational day (GD) 6 through 15 (organogenesis period). On GD 21, the dams were submitted to cesarean section. After macroscopic examination, the fetuses were removed from the uterus and subsequently cleared and stained with Alizarin Red. Then, the offprings were evaluated for skeletal variations and malformations. **Results and Discussion:** No external anomalies were found in the fetuses of all groups. Twelve types of deviation from the normal bone development were recorded. The most frequently encountered deviation in the offsprings of dams treated with EPPm were supernumerary and rudimentary ribs, bipartite sternebra and incomplete ossification of the parietal, interparietal, exoccipital, supraoccipital, zygomatic and palate. The incomplete ossification in the skull bones occurred with incidences of ≤ 1%. The incidences of rib and sternebra variations were < 25%. Less than 5% of fetuses showed absent cervical centrum.
Conclusion: This study demonstrates that there were neither treatment-or dose-related external signs of toxicity in any of the fetuses in any group, nor significant skeletal anomalies which could be directly related to EPPm administration.

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EX 21- EVALUATION OF FETAL SKELETAL DEVELOPMENT OF WISTAR RATS TREATED WITH *Pimenta pseudocaryophyllus* (GOMES) L.R. LANDRUM, CITRAL CHEMOTYPE

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Introduction: Medicinal plants have been used since ancient times as medicines for the treatment of a wide range of diseases. In this context, *Pimenta pseudocaryophyllus* (Myrtaceae), popularly known as “cataia” or “craveiro” in Brazil, has been widely used due to its antibiotic, hypotensive, antioxidant, diuretic and antinociceptive properties. Nevertheless, there are no studies regarding the use of this species during pregnancy.
Objectives: This study was performed to investigate the possible effects of the dry leaf extract of *P. pseudocaryophyllus*, citral chemotype, in the skeletal development of offsprings of rats treated during organogenesis.
Materials and Methods: Forty two pregnant female Wistar rats were divided into four groups: three treatment groups and a control group. The dry leaf extract of *P. pseudocaryophyllus*, citral chemotype was administered once daily by gavage to the dams at doses of 2000, 2500 or 3000 mg/kg from gestational day (GD) 6 through 15. On GD 21, fetuses were removed by cesarean section and examined for any morphological abnormalities. Then, fetuses were submitted to diaphanization processes and alizarin red staining. Using a stereomicroscope, the incidences of skeletal malformations and variations in the offsprings were recorded.
Results and Discussion: Fetal external examinations did not reveal any malformation or alteration. The most frequently encountered deviation in the offsprings of dams treated with *P. pseudocaryophyllus*, citral chemotype, were supernumerary and rudimentary ribs, bipartite, misshapen and incomplete ossification of sternebra. The incidences of rib and sternebra variations were < 30%.
Conclusion: The results showed that the dry leaf extract of *P. pseudocaryophyllus*, citral chemotype, in doses of 2000, 2500 and 3000 mg/kg/day administered during organogenesis period is not teratogenic for Wistar rats. Moreover, the fetal alterations observed in nearly all fetuses were not severe and occurred only in the axial skeletal.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 22- EVALUATION OF TWO CHEMOTYPES OF *Pimenta pseudocaryophyllus* (GOMES) L. R. LANDRUM ON BIOCHEMICAL PARAMETERS IN PREGANT RATS

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Introduction: The use of medicinal plants to treat a range of diseases is a natural therapy method, which is markedly present in Brazilian folk medicine. In spite of this disseminated practice, there are uncertainties regarding the safe use of many species. *Pimenta pseudocaryophyllus* (Myrtaceae), popularly known as "cataia" or "craveiro" in Brazil, is commonly used as a tranquilizer, menstrual and digestive regulator and in the treatment of cold symptoms. Its most common biological and pharmacological properties include antimicrobial, antinociceptive and anti-inflammatory, as well as anxiolytic-and antidepressive-like effects. The occurrence of two chemotypes, citral and *(E)*-methyl isoeugenol in *P. pseudocaryophyllus* confers changes in the composition of its essential oils and consequently possible differences in its therapeutic properties. As this species is a promising candidate to the development of a novel herbal medicine, toxicological studies are necessary to attest the safety of its use. **Objective:** This study aimed to analyze hepatic and renal biochemical parameters in pregnant rats treated with dry extracts of *P. pseudocaryophyllus* during organogenesis. **Materials and Methods:** Pregnant Wistar rats were treated during organogenesis period with dry leave extracts of *P. pseudocaryophyllus,* citral or *(E)*-methyl isoeugenol chemotypes, at doses 2000, 2500 or 3000 mg/kg/day. The control group received the vehicle (1% carboxymethylcellulose + 10% propylene glycol). On gestational day 21 (GD 21), the dams were anaesthetized and blood samples were collected through cardiac puncture. The serum was separated by centrifuging and then used for analysis of creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and albumin. **Results and Discussion**: The results showed that the serum levels of creatinine, urea, AST, ALT, total protein and albumin were not altered in the dams treated with the dry leaf extract of *(E)*-methyl isoeugenol chemotype when compared to the control group ($p>0.05$). On the other hand, a significant reduction in serum levels of ALP was observed in the dams treated with 3000 mg/kg of the dry extract of citral chemotype when compared to the control group ($p=0.03$). **Conclusion:** From a biochemical point of view, the dry leave extracts of two chemotypes of *P. pseudocaryophyllus* has not promoted substantial alterations in the serum levels of hepatic and renal markers. Furthermore, there was no correlation between the reduction in serum levels of ALP observed in the dams treated with 3000 mg/kg of citral chemotype and clinical signs of toxicity**.**

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 23- STUDY ON THE MATERNAL TOXICITY OF *Pimenta pseudocaryophyllus* (GOMES) L. R. LANDRUM, CITRAL CHEMOTYPE, IN WISTAR RATS

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Introduction: *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum is popularly known as "craveiro-do-mato", "louro-cravo" or "chá-de-bugre" in Brazil. This species has two different chemotypes: citral and (E) – methyl isoeugenol. Studies have shown that this medicinal plant presents antifungal, antibacterial, anxiolytic, hypotensive, antinociceptive and anti-inflammatory properties. In addition, its leaves are used in the preparation of medicinal teas to treat colds as well as to regulate digestion and menstruation. Although the therapeutic applications of *P. pseudocaryophyllus* have been described, there is no information about the safety in using this medicinal plant during pregnancy. **Objective:** This study was performed to investigate the reproductive toxicity of the dry leaf extract of *P. pseudocaryophyllus*, citral chemotype, in Wistar rats. **Materials and Methods:** Pregnant Wistar rats were orally treated with the vehicle or the dry leaf extract of *P. pseudocaryophyllus*, citral chemotype, at dose levels of 2000, 2500 or 3000 mg/kg/day from gestational day (GD) 6 to 15. The dose levels were selected on the basis of available data from our previous studies. Body weight gain, food and water consumption were recorded daily throughout the gestation. On GD 21 cesarean section was performed. The uterus was removed and inspected for resorption and fetal deaths. Each live fetus was weighed and received a gross external morphologic examination. Subsequently, the dams were examined for standard parameters of reproductive outcome. **Results and discussion:** During organogenesis period (GD 6-15), the dams treated with the dry leaf extract of *P. pseudocaryophyllus*, citral chemotype, showed a reduction in weight gain as well as in the average feed intake when compared to the control group ($p<0.05$). There were no significant differences in water consumption between groups during pregnancy ($p>0.05$). Moreover, no significant differences between groups ($p> 0.05$) regarding reproductive parameters were observed such as total number of fetuses, live and dead fetuses, number of implantation sites, resorptions and corpora lutea, fetuses and placenta weights. Furthermore, there were no external abnormalities in the offsprings of any treatment groups. **Conclusion:** This study demonstrates that the dry leaf extract of *P. pseudocaryophyllus*, citral chemotype, when administered to pregnant Wistar rats at the doses 2000, 2500 or 3000 mg/kg/day causes maternal toxicity, especially in the organogenesis period. On the other hand, there were no toxicological effects of the treatment on the reproductive performance of the dams.

Acknowledgments: FAPEG and PROBIP - UEG for financial support. PIBIC-AF/CNPq (Process nº 153840/2013-7) and PBIC/UEG.

EX 24- EFFECT OF THE AQUEOUS EXTRACT OF *Eugenia punicifolia* (MYRTACEAE) ON THE PHARMACOKINETICS OF MIDAZOLAM IN RATS

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Introduction: The utilization of natural products for the treatment of different diseases has gained attention due to the possibility of appearance of drug interactions with allopathic medicines. According to the literature, pharmacokinetic mechanisms are the among the most important and discussed ways related to drug-natural product interactions, especially involving oxidative metabolism by cytochrome P450 enzymes and the outcomes prompted by these interactions were both poorer efficacy and unusual toxicity caused by the drug. The leaves of the herb *Eugenia punicifolia*, popularly known by the local inhabitants by pedra-ume-caá, are regularly used in the Amazon region in the treatment of pain, inflammation and diabetes. However, there are few studies regarding its efficacy and safety. **Objective:** The aim of this research was to investigate the effects of the aqueous extract of *E. punicifolia* on the pharmacokinetics of midazolam in rats. **Materials and methods:** Leaves of *E. punicifolia* were collected at the Section of Medicinal Plants of Empresa Brasileira de Pesquisa Agropecuária in Manaus, Brazil. A standard fresh aqueous extract of the herb at the concentration of 7.5% (p/v) was used throughout the study. Before experiment, anesthetized male Wistar rats were subjected to a surgical procedure to install a cannula in the left carotid artery to enable serial blood drawings. Individuals were allocated in 3 groups (n=6) and received different doses of extract (vehicle, 55 mg/Kg and 550 mg/Kg) by oral gavage. A single oral dose of midazolam (20 mg/Kg), a CYP3A marker in rats, was administered to all animals. The plasma levels of midazolam were monitored using a validated UV-HPLC method and pharmacokinetics parameters were obtained by PKSolver software. The study was approved by Ethics Committee on the Use of Animals of Universidade Federal do Amazonas (protocol number 073/2012). Analysis of variance was employed to verify statistical differences among groups ($p < 0.05$). **Results and discussion:** The low dose group (55 mg/Kg) had plasma Cmax and AUC 2.3 and 1.4-fold higher and clearance 40% lower when compared to vehicle group (Low dose group - Cmax: 3.64 µg/mL; AUC: 2.39 µg.h/mL; Cl: 0.002 L/h/kg; Vehicle group - Cmax: 1.58 µg/mL; AUC: 1.65 µg.h/mL; Cl: 0.003 L/h/kg). On the other hand, no differences were observed between vehicle and high dose group (550 mg/Kg - Cmax: 1.14 µg/mL; AUC: 1.68 µg.h/mL; Cl: 0.003 L/h/kg). **Conclusions:** The single oral administration of an aqueous extract of *E. punicifolia* at the dose of 55 mg/Kg caused a marked effect on the pharmacokinetics of midazolam in rats, possible owing to the enzymatic inhibition of CYP3A. Further studies should be done to clarify the results obtained.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 25- ALTERATION OF THE PHARMACOKINETICS OF THEOPHYLLINE BY *Paullinia cupana* (SAPINDACEAE) IN RATS

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Introduction: The seeds of *Paullinia cupana* (Sapindaceae) have been popularly used to prepare different beverages by the Amazonian inhabitants for a long time ago mainly due to its stimulant properties. Therefore, several studies have been carried out in order to investigate its efficacy regarding the attributed pharmacological activities. On the other hand, few reports have focused on its toxicological profile. In this context, the effect of natural products on the activity of CYP enzymes is of paramount importance since these proteins are involved in the metabolism of several drugs. Among CYP enzymes, CYP1A is one of the most studied through in vivo assays using drug probes, such as theophylline. **Objective:** The aim of this research was to investigate the effects of the aqueous extract of *P. cupana* on the pharmacokinetics of theophylline in rats. **Materials and methods:** Individuals were allocated in three groups (n=6) and received different once daily doses of extract (vehicle, 82.1 mg/Kg and 821 mg/Kg) by oral gavage during two weeks. Before experiment, anesthetized male Wistar rats were subjected to a surgical procedure to install a cannula in the left carotid artery to enable serial blood drawings. All animals were administered a single oral dose of theophylline (10 mg/Kg), a CYP1A marker in rats. The plasma levels of theophylline were monitored using a validated UV-HPLC method and pharmacokinetics parameters were obtained by PKSolver software. The study was approved by Ethics Committee on the Use of Animals of Universidade Federal do Amazonas (protocol number 073/2012). Analysis of variance was employed to verify statistical differences among groups ($p < 0.05$). **Results and discussion:** Animals subchronically treated with the aqueous extract of *P. cupana* (AUC: 1.19 and 0.34 mg.h/mL for 82.1 and 821 mg/Kg, respectively) had significantly lower exposition to theophylline than controls (AUC: 3.53 mg.h/mL). On the other hand, drug clearance was statistically higher in treated individuals (Cl: 2.44 and 7.27 L/h/kg for 82.1 and 821 mg/Kg, respectively) than controls (Cl: 0.71 L/h/kg). **Conclusions:** The multiple oral administration of an aqueous extract of *P. cupana* caused a marked effect on the pharmacokinetics of theophylline in rats, possible owing to the enzymatic induction of CYP1A. Further studies should be done to clarify the results obtained.

EX 26- TOXICITY EVALUATION OF *Psychotria colorata* CRUDE EXTRACT DURING ORGANOGENESIS AND FETAL DEVELOPMENT IN WISTAR RATS

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Introduction: *Psychotria colorata* (Rubiaceae) is a plant used in traditional medicine for treatment of ear and abdominal pains. It is found in Venezuela, Guyana and Brazil, in the North, Northeast and Midwest regions. Brasilian farmers reported that the plant promotes abortions in cows. **Objective:** Evaluate the effects of administration of *P. colorata leaves* crude extract (EB) in Wistar rats during organogenesis and fetal development period. **Material and methods:** The leaves of the plant were collected in the Carmolandia city, Tocantins State, Brazil, dried at 55°C, percolated in ethanol and concentrated in rotary evaporator at 45°C to obtain crude extract (EC). Rats were randomly divided into experimental group (n = 12), treated with EC diluted in 5% Tween 80, and control group (n = 10) that received only the vehicle. Treatments were administrated by gavage from 6th day of gestation (GD6) up to the GD20, according to the protocols of OECD 414[1] and EPA 1996[2]. To simulate natural conditions of intoxication, the dose of EC, 2178 mg/kg body weight, corresponded to what would be consumed if the plant was only source of food. The experimental protocols adopted were approved by the Ethics Committee under No. 23101.000666/2014-39. During the experimental period the water and feed intake and body weight were daily monitored. On GD21 animals were euthanized, and uterine horns were weighted, number of corpora lutea, dead and live fetuses, implantations, resorption sites were counted, preimplantation and postimplantation losses were calculated. Aditionaly, for each fetus, the placenta and body weight were recorded, and fetal crown-rump length was measured. **Results and discussion:** The experimental females showed an increase in food consumption in a single day. However, no interference was detected in the weight gain of the females. In addition, there were no significant differences in water consumption, body weight and reproductive parameters of experimental animals compared to the control group. Phytochemical analysis of *P. colorata*, identified the presence of pyrrolidinoindoline alkaloids, hodgkinsine, chimonanthine, quadrigemine C and psychotridine and the quinoline alkaloids, calycanthine and isocalycanthine. It is known that compounds with different classes or structures may perform the same activity or antagonistic activity in which a compound can inhibit the action of another, resulting in no toxicity during the treatment period. **Conclusion:** Our results indicate that consumption of EC of *P. colorata* leaves during organogenesis and fetal development period did not cause reproductive alterations in female rats.

References:

1. Organiz. for Econ. Coop. and Develop. (2001) First addendum to OECD guideline 414 for testing of chemicals, "Prenatal Developmental Toxicity Study." OECD, Paris, pp. 1-11.
2. U.S. Environ. Protec. Ag. (1996) Health Effects Test Guidelines OPPTS 870.3800: Reproduc. and Fertility Effects (Draft). Fed. Reg. 61(212):56274-56322.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 27- SERUM BIOCHEMICAL EVALUATION OF FEMALE WISTAR RATS TREATED WITH *Psychotria colorata* ALCALOIDIC EXTRACT DURING PREGNANCY AND LACTATION

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Introduction: *Psychotria colorata* is a plant of the family Rubiaceae, commonly known as Perpétua do mato and repolho. The plant is used medicinally for pain-related purposes among Amazonian caboclos in Brazil. Pharmacological profile from extracts of *P. colorata* suggests opioid-like analgesic activity attributed to pyrrolidinoindoline alkaloids such as hodgkinsine and quadrigemine C. **Objective**: Investigate the effects of administration of *P. colorata* leaves alcaloidic extract (EAL) during gestation and lactation in Wistar rats on serum biochemical parameters. **Material and methods:** The leaves of the plant, collected in the Carmolândia city, Tocantins State, Brazil, were dried at 55°C, moistened with NH4OH 6N, extracted with ethanol in a Soxhlet apparatus and concentrated in rotary evaporator at 45°C to obtain alcaloidic extract (EAL). Female rats were randomly divided into experimental group (n = 12), treated with EAL diluted in 5% Tween 80, and control group (n = 12) that received only the vehicle. Treatments were administrated by gavage from 6th day of gestation (GD6) up to the post natal 21 (PND 21), in agreement with the protocols of EPA 1996[1] and OECD 426[2]. The dose of EAL, 226 mg/kg body weight, corresponded to an exclusive consumptionof the plant, to simulate natural conditions of intoxication. The experimental protocols adopted were approved by the Ethics Committeeof Federal University of Tocantins under No. 23101.000666/2014-39. During thetest period, mortality, clinical signs, food and water consumption, changes in body weightwere recorded. The serum biochemical parameters, ALT, AST, albumin, bilirubin, cholesterol, glucose, creatinine, triglycerides and uric acid were determined. **Results and Discussion:** No mortality and clinical signs of toxicity were observed in experimental groups. Additionally, no significant differences were detected in the food and water consumption and body weight of rats treated with the EAL compared to the control group. There was increase in level of uric acid in serum of experimental rats relative to control group; others biochemical parameters evaluated in this study were not affected by the treatment. The increase in uric acid might be due to the possible action of alkaloids of *P. colorata* on kidneys of rats treated. In fact, administration of opioids substances such as morphine, promote increase in uric acid in serum of rats due to damage caused in the kidney[3]. **Conclusion:** Consumption of EAL during gestation and lactation promote an increase in serum uric acid in rats.

References:

1. U.S. Environ. Protec. Ag. (1996) Health Effects Test Guidelines OPPTS 870.3800: Reproduc. and Fertility Effects (Draft). Fed. Reg. 61(212):56274-56322.
2. Organiz. for Econ. Coop. and Develop. (2007) First addendum to OECD guideline 426 for testing of chemicals, “Developmental Neurotoxicity Study.” OECD, Paris, pp. 1-26.
3. Sumathi, T.; Devaraj, S. N. Effect of *Bacopa monniera* on liver and kidney toxicity in chronic use of opioids. Phytomedicine 16: 297, 2009.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 28- GENOTOXICITY AND MUTAGENICITY INDUCED BY ACUTE CRACK-COCAINE EXPOSURE IN MICE

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Context. It is well known that substances used to human consume might have genotoxic and/or mutagenic potential such as tobacco and alcohol. Crack cocaine is an illicit drug derived from cocaine, in which use and abuse have increased around the world, especially in developing countries. **Objectives.** The aim of this study was to evaluate genomic damage in multiple organs of mice following acute exposure to crack cocaine using a unique injection of three different dosages. **Material and Methods.** A total of twenty (20) C57BL/10 mice were distributed into four groups (n=5), as follows: 0; 4.5; 9 and 18 mg/kg b.w. of crack-cocaine by intraperitoneal route (i.p.). All animals were sacrificed 24h after i.p. injection. Peripheral blood, brain, liver cells and kidney cells were processed by a technique named assay Comet Assay or single cell gel electrophoresis. Bone marrow blood and liver cells were processed for micronucleus count. **Results.** Results obtained using Comet Assay technique showed genotoxic alterations in the Tail Moment values of the CRACK 18 group compared to the control group in blood and brain tissues ($p<0,05$). Liver and kidney did not present genetic damage for all concentrations tested. Concentrations 4,5 mg/kg and 9 mg/kg did not cause any DNA damage in the analyzed tissues. The number of micronucleated cells did not increase after crack cocaine exposure in bone narrow or liver cells. **Conclusion.** Therefore, we concluded that the Maximum dosage of 18mg/kg of crack cocaine is a genotoxic agent. Cerebral and blood cells demonstrated to be more sensitives to the acute exposure. Dosages tested were not able to induce mutagenicity in liver cell and bone marrow tissues in the analyzed period.

Key words: crack cocaine; genomic instability; DNA damage; mice

EX 29- TOXICITY ASSESSMENT OF *Copaifera langsdorffii* OIL-RESIN (COPAIBA) AGAINST *Artemia salina*

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Introduction: The popular use of medicinal plants is common in Brazil, often without proper guidance, which can turn a natural product useful for health into a problem. Used as anti-inflammatory[1], the *Copaifera langsdorffii* (Copaíba) has its frequent use by the population. Trees "copaibeiras" are popularly known as copal, podóia or pau d'óleo, which belong to the genus *Copaifera* (Leguminose-Caesalpiniaceae). The oil present in the *C. langsdorffii* is a transparent liquid, reddish formed of resin acids and volatile substances. Thus, studies about safety of its use should be better established. **Objective:** The aim of this study was to identify the concentration of the resin obtained from *C. langsdorffii* is effective lethality on brine shrimp equivalent of LC50. **Methods:** The oleoresin of the copaiba was obtained in the Araripe National Forest, the city of Barbalha, Ceará, through perforations in its trunk. The toxicity study was conducted on *Artemia salina* in a solution of sea salt at a concentration of 30g L^{-1}, which must have a pH between 8.0 and 9.0. If the solution does not present a suitable pH, adjusted to pH 0.1 with brine mol L-1 NaOH. This seawater solution was used for hatching brine shrimp eggs and preparing the other dilutions. The eggs were placed to hatch in the solution for 48 hours with aeration and constant lighting the temperature 25°C. Having these hatched prepared solutions containing saline solution and samples to be tested with the oleoresin *C. langsdorffii* at concentrations of 1000, 500, 250, 125, 50, 10, 5 and 1 ppm concentrations where each received 10 *Artemia salina* larvae. This assay was performed in triplicate samples, and the count of living and dead animals carried out after a 24-hour period, aiming to find the LC50 of this oil-resin. **Results and Discussion:** Concentrations of less than 1µg/mL had potential fatal toxicity to approximately 50% of larvae, showing a high potential toxic to the oleoresin since lethal concentrations below 10^3 mg/mL indicate a potential toxic activity [1]. **Conclusion:** Exposure to oil-resin of *C. Langsdorffii* presented a high toxic potential and may pose a toxic risk, further tests are needed to investigate the targets and mechanisms of toxicity.

Reference

1. MEYER, B. N. et al. Brine shrimp: a convenient general bioassay for active plant constituents. **Planta Med,** v. 45, n. 5, p. 31-4, May 1982.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 30- CHRONIC EXPOSURE TO INORGANIC MERCURY CAUSES COGNITIVE AND MOTOR DEFICITS RELATED TO CITOTOXICITY AND CELL DEATH BY APOPTOSIS IN MOTOR CORTEX AND HIPPOCAMPUS OF ADULT RATS

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Introduction: Mercury is a highly toxic heavy metal, which can be found in organic and inorganic elemental forms in the environment. In localities contaminated by this metal, mercury exposure usually occurs in low daily doses over a long period. The inorganic mercury has lower liposolubility and consequently, lower absorption in the body, and lower passage through the blood brain barrier. For this reason, exposure models using inorganic mercury in rats to evaluate its effects in the central nervous system are rare, mainly in adults. **Objective:** This study investigated the potential of low concentration of Mercury chloride ($HgCl_2$) to promote motor and cognitive changes and to trigger cytotoxicity and cell death by apoptosis in the motor cortex and hippocampus in a chronic exposition model. **Materials and Methods:** 30 rats were exposed for 35 days to a dose of 0.375 mg/kg/day. After this period, the animals were submitted to a series of motor and memory behavioral tests: open field, beam walking, rotarod and inhibitory avoidance. After behavioral tests, the animals were sacrificed. Motor cortex and hippocampus were collected for measurement of total deposited mercury using an atomic absorption spectrometry. Assessment and quantification of cytotoxicity and apoptosis were made using Cytotox-glo™ e Apotox-glo® systems (Promega), quantitated by fluorescence and luminescence, respectively. All data were tabulated and statistically analyzed for normality (Shapiro-Wilk test) and differences between the samples (Student's t test), assuming $p < 0.05$. **Results and Discussion:** Chronic exposure to inorganic mercury caused a reduction in horizontal and vertical spontaneous locomotor activities observed by the open field test; beam and walking and rotarod test indicated impaired balance and fine motor skills; decreased short and long-term memory were verified by the inhibitory avoidance test. Furthermore, we found that this exposition model led to cytotoxicity and cell death by apoptosis induction in the motor cortex and hippocampus, in addition to the formation of deposits of mercury in these regions. **Conclusions:** Low inorganic mercury concentrations were able to cross the blood brain barrier in adult rats, depositing in the neural parenchyma, and inducing cell death by both cytotoxicity and apoptosis in the hippocampus and motor cortex, hence promoting cognitive and motor deficits. Our study demonstrated that inorganic mercury can promote neurological deficits and cell death in the central nervous system, even when adult organisms are chronically exposed to low mercury concentrations.

EX 31- METHYLMERCURY CRONIC EXPOSURE EFFECTS ON MOTOR PERFORMANCE AND BIOCHEMICAL PARAMETERS IN THE MOTOR CORTEX OF RATS

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Introduction: Due to the increasing bioaccumulation of methylmercury (MeHg) in the marine food chain, the human consumers are exposed to low doses of MeHg continuously through the consumption of fish and seafood. However, the effects of chronic exposure to low doses of MeHg in the motor cortex during adult life related to motor performance and biochemical changes are little known. **Objective:** This study investigated whether MeHg, in a model of chronic exposure at low concentration, is capable of promoting motor changes and oxidative stress in the motor cortex of adult rats. **Materials and Methods:** Fifteen Wistar rats were exposed for MeHg at a dose of 0.04 mg/kg/day during 60 days by intragastric gavage. Fifteen other animals (control) received only oil vehicle by gavage. After this period, the animals were submitted to a sequence of behavioral motors tests with open field, beam walking and rotarod test. After intoxication, the animals were sacrificed and motor cortex collected for measurement of total mercury deposited in the nervous tissue by atomic absorption spectrometry. The levels of nitrites present in the samples were measured. All data were tabulated and statistically analyzed for normality (Shapiro-Wilk test) and difference between the groups (Student's t test, $p < 0.05$). **Results and Discussion:** Our results demonstrated that chronic exposure to MeHg induced reduction in horizontal and vertical spontaneous locomotor activity, observed by the open field test; increased the time of performance on the square and circular beams in the beam walking test; decreased latency and increased the number of falls in the 3 rotarod exposures. Indeed, we observed that chronic exposure to MeHg promoted deposits of mercury in the neural parenchyma, as well as trigger oxidative stress, elevated levels of nitrites. Therefore, our work demonstrated for the first time that even in adult organisms with a low daily dose exposure, MeHg might trigger poor locomotor performance and motor coordination and balance impairment in addition to the increase of pro-oxidant marker in the motor cortex. **Conclusions:** Our data demonstrates that MeHg chronic exposure, in this proposed model, promoted deposits of total mercury in the motor cortex, which may be associated with mechanism of oxidative stress diagnosed, related to the loss of motor abilities observed in animals intoxicated.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 32- PRECLINICAL SAFETY PHARMACOLOGY STUDY OF A PROTEIN ISOLATED FROM *Morinda citrifolia* L. SEEDS WITH POTENTIAL FOR INFLAMMATORY PAIN RELIEF

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Introduction: Noni (*Morinda citrifolia* L., Rubiaceae) is a medicinal plant that has been reported to have a broad range of health benefits. Recently, our research group carried out the isolation and characterization of a lipid transfer protein, named *Mc*LTP$_1$, from *Morinda citrifolia* L. seeds with potential to relieve inflammatory pain. **Objective:** This study was designed to evaluate safety pharmacology of *Mc*LTP$_1$ in preclinical environment using mice as experimental model. **Materials and Methods:** *Mc*LTP$_1$ was isolated from noni seed extract as previously described. Acute oral toxicity study was carried out according to OECD guideline 423. Nine female mice (25-30 g) were divided into three groups i.e., control (NaCl 0.15 M as vehicle 10 mL/kg) and two experimental groups (8 mg/kg and 80 mg/kg *Mc*LTP$_1$). Animals were observed for their mortality and clinical signs of toxicity at 30 min, 1, 2 and 4 h and thereafter once a day for 14 days following vehicle and *Mc*LTP1 administration. The 28-day repeated oral toxicity was performed according to the OECD guideline 407 and *Mc*LTP$_1$ was administered by oral route at the dose of 8mg/kg/day. Mice of both sexes were divided in groups with 10 animals (5 males + 5 females) in each. Control group received NaCl 0.15 M as vehicle. At the end of the stipulated treatment period, blood samples were collected for haematological and biochemical analysis. Necroscopy was done in all the animals on 29th day and the organs were observed for histopathological investigation. In addition, the potential allergenicity of *Mc*LTP$_1$ after oral route of sensitization was evaluated. Mice (06 per group) were sensitized orally with *Mc*LTP$_1$ 10 and 50 µg for 10 days and blood samples were collected on days 7, 14, 21, 28, 35 and 42 days after first sensitization for measurements of allergen-specific serum IgG and IgG1 levels. Animal experimental procedures were approved by the Committee for the Ethical Use of Animals of Federal University of Ceará (CEUA - UFC no. 37/13). **Results and Discussion:** *Mc*LTP$_1$ did not induce significant changes in mice's gross behavior and did not caused mortality in any of the animals tested throughout the experimental period. Mice body weight as well as hematological, biochemical and histopathological parameters were not affected by oral administration of *Mc*LTP$_1$, 8 mg/kg or 80 mg/kg (p.o.). However, increased and significant ($p<0.05$) levels of IgG and IgG1 were observed in mice sensitized with *Mc*LTP$_1$ suggesting that this protein, similar to other LTPs, is potentially allergenic to mice. **Conclusion:** The results presented contribute to the future use of *Mc*LTP$_1$ as a biopharmaceutical, demonstrating that the protein is considered safe according to OECD toxicity parameters but has the potential to act as true food allergen.

EX 33- ASSESSMENT OF POTENTIAL NUTRACEUTICAL EXTRACT OF PURPLE CARROT (DAUCUS CAROTA L. SSP. SATIVUS VAR. ATRORUBENS ALEF.) IN MULTIPLE ORGANS (PERIPHERAL BLOOD, BONE MARROW AND LIVER) IN RATS EXPOSED TO CADMIUM CHLORIDE.

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In recent years, concern for the environment and health become increasingly worrisome and emerging due to the deposition of toxic substances in water, soil and air. The metals are causing changes in the quality of soil, water and air, as well as ecological impacts for its ability to incorporate into tissues and then be assimilated into the food chain. However, cadmium, and lead and mercury, metal is not essential and is toxic even in small quantities and bioaccumulation potential in living beings, causing oxidative stress and injuries in various organs such as liver, kidney, lung, and others. Upon this metal contamination of the alternatives recently researched are the benefits found in functional foods, that is, in addition to its nutritional qualities have nutraceutical effects mainly on their antioxidant activities. The dye purple carrot extract has been studied for its antioxidant properties, due to the high polyphenol content. Therefore the aim of this study is to evaluate the effects of purple carrot extract in multiple organs of rodents exposed to cadmium chloride such as liver and peripheral blood and bone marrow. To analyze the genotoxicity was used comet assay, mutagenicity the micronucleus test in bone marrow blood, immunohistochemistry for expression 8OHdG the evaluation of oxidative stress, gene expression of oxidative stress enzymes (catalase, and Cu, ZnSOD MnSOD) and apoptosis marker (cytochrome C), and histopathological examination of the liver of animals. For this study, 20 Wistar rats being subdivided into n:. 5 and Ctrl group (untreated) group cadmium (CD), cadmium-cen.1 group (CD + CEN.1) dose 200mg dose I: 0.5 mg / kg / day, cadmium carrot group 2 (CD + CEN.2) 400mg dose II Dose: 1.0 mg / kg / day. The treated animals received an intraperitoneal injection of cadmium chloride (1.2 mg / kg body weight) diluted in water and, after 15 days group (CD + CEN.1) received via the trough carrot extract dose 1 diluted in 500ml of water and the group (CD + CEN.2) received the same treatment with the dose 2. Both carrot extract doses were able to decrease the mutagenic and genotoxic effects induced by cadmium cells in the peripheral blood, bone marrow bone and liver, as represented by the comet and micronucleus assays. A decreased expression of anti-8-hydroxy-20 - deoxyguanosine (8OHdG) in hepatocytes of animals exposed to cadmium and treated with the extracts was also detected. Higher CuZn-SOD activity was observed in liver cells in both groups treated with dye extract. Significant differences were observed in relation to Mn-SOD activity between the treated groups and reduction of catalase between groups. A remarkable increase of cytochrome c from both groups treated with dye before the cadmium group. Histopathological analyzes showed an evident reduction of tissue damage in the liver in both groups treated with doses of carrot. The set of our results shows that both doses of the dye to purple carrot extract base were able to exercise clear protective activity, antimutagenic and antigenotoxic in blood and liver cells and preservation of tissue structures to the rat liver lesions exposed to cadmium.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 34- THE HERBICIDES TRIFLURALIN AND TEBUTHIURON FAIL TO ACTIVATE THE NRF2 ANTIOXIDANT RESPONSE

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Introduction: Herbicides comprise a heterogenous category of chemical products designed to control weeds. Trifluralin and tebuthiuron are commonly used in Brazil to minimize weed growth in sugar cane crops. Some herbicides are described as selective in their mechanism of action, however, they can reach off-target organisms and cause harmful effects. Some herbicides are known to induce oxydative stress in humans and can lead to activation of the transcription factor Nrf2 and the antioxidant response pathway. Under conditions of oxidative stress, Nrf2 is activated and regulates the expression of various antioxidant genes, such as NQO1. **Objective:** The objective of the study was to analyze the potential of trifluralin and tebuthiuron to activate the Nrf2 signaling pathway. **Material and Methods:** HepG2 cells were exposed to a wide range of Trifluralin and Tebuthiuron concentrations and the MTT assay was conducted to evaluate cell viability. The concentrations of each herbicide tested in the MTT assay were 1, 5, 10, 20, 50 and 100 uM. qRT-PCR was performed to analyze the expression of NQO1 following Nrf2 activation using either a positive control compound known to activate Nrf2 (AI-1) or trifluralin and tebuthiuron. In this assay, it was used 5, 10, 20, 50, and 100 uM of trifluralin and 100 uM of tebuthiuron. Luciferase reporter contructs contaning the Nrf2 DNA binding motif (antioxidant response element, ARE) were utilized to directly measure the ability of trifluralin and tebuthiuron to activate Nrf2 and values were compared to the positive control Nrf2 activator (AI-1). The concentrations of each herbicide tested in the luciferase assays were 10 and 100 uM. Statistical analysis was done using ANOVA test followed by Dunnett. **Results and Discussion:** MTT assay showed a decrease of more than 30 % in cell viability of HepG2 exposed to 50 and 100 uM of trifluralin, suggesting a harmful effect of trifluralin on cell viability at these higher concentrations. However, the qRT-PCR showed no influence in expression of NQO1 and the reporter assay showed no activation of Nrf2 at any concentration tested, indicating that trifluralin does not activate Nrf2 and trigger the antioxidante response in HepG2 cells. Tebuthiuron yielded negative results for all tests indicating no toxicity or Nrf2 activation in HepG2 cells. **Conclusion:** Neither herbicides, at the high micromolar concentrations tested, activated the Nrf2 signaling pathway in HepG2 cell cultures. Although not an Nrf2 activator, high concentrations of Trifluralin did prove to be toxic to human HepG2 cells, and the mechanism of toxicity needs to be further explored.

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EX 35- LEAD AND CADMIUM METALS MIXTURE ON MEMORY BEHAVIOR: IS ALWAYS A MIXTURE MORE TOXICANT THAN THE INDIVIDUAL SUBSTANCES?

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Introduction: Neurotoxicity of individual metals is well investigated but the effects of metals mixture, an environmental reality, on learning and cognitive development remains scarce and unclear. Particularly, a mixture of lead (Pb) and cadmium (Cd) is among the major toxic agents found in environment, and importantly the exposure to these metals during the development of central nervous system is correlated with behavioral and cognitive changes that persist into adulthood. **Objective:** To investigate if the effect of Pb and Cd mixture is more toxic than the individual metals on the memory behaviors in young and adult offspring. **Material and Methods:** Adult female Wistar rats were divided into four groups and received daily: drinking water as vehicle (Group 1: Control), individual metals (Group 2: 350mg/L of Pb acetate and Group 3: 10mg/L of Cd acetate), or mixture (Group 4: Pb+Cd). The treatment of the animals started from gestation day 1 (G-1) until the pups weaned (postnatal day-21, P-21). To eliminate confounding consequences of the female reproductive cycle, only male offspring was used for the behavioral study. During offspring development, in young (P-30) and adult (P-80) were investigated the memory behaviors using the novel object recognition and radial eight arms maze apparatus. The novel object recognition test is used to evaluate short- and long-term cognitive memory, whereas the radial eight arms maze is used to analyze spatial memory. The data were subjected to ANOVA followed by Tukey test ($p<0.05$). **Results and Discussion:** Short-term memory was unchanged in young animals; however in the same animals the long-term memory was significantly damaged by the Pb alone compared to Control group. Despite spatial memory did not vary significantly in young animals, the most harmful effect of Pb was evident in adulthood when compared to other groups. Although the Pb affected the cognitive memory in young rats, this effect disappeared in adulthood exactly when this metal alone severely impaired spatial learning. These findings showed that most deleterious effects on memory behavior were provoked by Pb alone suggesting that individual substances seemed to be more toxic during the period of the central nervous system development. Notably, these changes remained in adulthood indicating that the *imprinting* effect may be occurred. **Conclusion:** We suggest that when exposure occurs during pregnancy the effects of the individual substances seemed to be more toxic than these substances associated in a mixture. The mechanism action and a possible competition for binding sites may be investigated.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 36- CYTOTOXIC AND HEMOLYTIC ACTIVITY METHANOL EXTRACT OF *Hura crepitans* L. (Euphorbiaceae) LATEX

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Introduction: *Hura crepitans* L., popularly called as "Assacu", is a large tree native from South America, particularly from the Amazon region. The "Assacu" latex has toxic effects when in contact with skin and mucous membranes and it's used by locals for river fishing to stun or killing the fishes. **Objective:** This study aims to evaluate the cytotoxic and hemolytic activity of the methanol extract of *Hura crepitans* L. **Materials and Methods:** *Hura crepitans* L. latex samples were collected in the city of Coari, Amazonas, Brazil. Subsequently, exhaustive maceration was performed with ultrasound assistance, using methanol as solvent. The cytotoxic activity was evaluated by the Alamar Blue test using a strain of human fibroblasts (MRC-5) and a melanoma cell line (SK-Mel 3). The concentrations tested were 150, 125, 100, 75, 50, 25, 12.5 ug/ mL, diluted in sterile DMSO (negative control), incubated and evaluated every 24 hours for 72 hours. For hemolysis assay, the samples were diluted in DMSO and distributed into 96 wells plate at concentrations of 1000, 500, 250, 125, 62.5 and 31.25 ug/mL using saline as negative control and 0.5% Triton as positive control. The samples were treated with a 2% erythrocytes solution obtained from Wistar rats. The plate was maintained under constant agitation, centrifuged and the supernatant was read in an ELISA plate reader to determine the hemolytic activity. The statistical analyzes were performed utilizing GraphPadPrism 6. **Results and Discussion:** Related to the hemolytic activity, the EC50 (µg /ml) couldn't be calculated, due to the highest concentration tested has been 45.04 ± 5.42% hemolysis. Related to the cytotoxic activity was obtained the following results to the lines MRC-5 = 140.9 µg/mL and SK-Mel 3 = 21.99 µg/mL. **Conclusion:** The methanol extract of *Hura crepitans* L. didn't show significant hemolytic activity at the highest concentration tested (1000 µg/mL), revealing a degree of certainty as to the use of this plant species. The extract showed significant cytotoxic activity against malignant cell line SK-Mel 3, and lower activity against the normal cell line MRC-5 suggesting selectivity as its lethality. The low hemolytic activity in erythrocytes suggests that the cytotoxicity of the extract isn't related to membrane damage, this activity may be related to another mechanism of action such as, apoptosis.

Keywords: *Hura crepitans* L., hemolysis, cytotoxicity, methanol extract

EX 37- ACUTE TOXICITY AQUEOUS EXTRACT OF PLANT SPECIES *Myrcia guianensis* (MYRTACEAE)

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Introduction: The use of medicinal plants showed over the years that certain plants have potentially dangerous substances. The tea leaves of pedra-ume-caá (*Myrcia guianensis* - Myrtaceae) is widely used by the Amazonian population and indicated for inflammation, colds and diabetes. However, there are no studies that evaluate the possible toxic effects**.**
Objective: The aim of the study was to evaluate the acute toxicity of the aqueous extract (AE) Myrcia guianensis in an animal model.
Materials and Methods: AE was prepared from the powder of dried leaves of *Myrcia guianensis* (1: 7 w / v). To evaluate the toxicity Swiss mice of both sexes were used (n = 3 ♂ and 3 ♀ per group) following the methodology Richter & Campbell (1967). The doses administered to each group were 1, 2, 4 and 5 g / kg (po) to fasted animals for 10h. Behavioral responses were observed within the first 6 hours, the 8th and 12ªh and for 14 days after the administration of AE. On the 14th day the animals were necropsied for analysis of liver, spleen and kidneys. The data obtained from the organ weights were expressed as mean ± SD. This study was approved by CEUA-UEPA (Protocol N° 043/2011).
Results and Discussion: During the experimental period there was no death of any animals due to the AE administration of the tested plant. The clinical signs were absent for all doses. In the literature there is reference to the greater sensitivity of female animals, but this study wasn't observed differences between sexes. The kidneys and liver are responsible for metabolism and excretion of xenobiotics, while the spleen provides signs of toxicity to the immune system of animals, but the autopsy didn't show significant macroscopic changes in organ weights of the test groups compared to the control group suggesting low or lack of toxicity of the test species.
Conclusion: Thus, according to the results obtained, AE *M. guianensis* has low acute toxicity exposure. However, tests that evaluate use of repeated doses should be made to ensure safety in their use.

References: CAMPBELL, D.E.S.; W. RICHTER. Viewpoints on the Digital Computer Treatment of Biological Experimental Data. **Acta Pharmacologica et Toxicologica**, v 25, p 345-63, 1967.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 38- EVALUATION OF THE EFFECTS OF PCB ON HEMATOLOGICAL PARAMETERS IN RATS

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Introduction: Polychlorinated biphenyls (PCBs) are synthetic chemicals, persistent in the environment, that enter food chain and bioaccumulate. Experimental studies have shown that PCBs can induce different adverse effects such as acne-like skin conditions, liver disorders, immune function reduction, and impaired reproduction and thyroid function. Although investigated, hematological changes do not appear to be well-characterized effect of PCB exposure. **Objective:** The aim of this study was to evaluate the effects of subacute exposure to PCBs given in six different doses, from low ones that correspond to the exposure of the general population to moderate and high doses, on the count of red blood cells (RBC), white blood cells (WBC), platelets (PLT) and hemoglobin content (Hb) in rats. **Materials and Methods:** Six groups (n=6-7 per group) of the adult male Wistar rats were orally exposed to Aroclor 1254 dissolved in corn oil at the doses of 0, 0.5, 1, 2, 4, 8, 16 mg PCBs/kg b.w./day for 28 days. At the end of experiment, rat peripheral blood samples were taken and examined by flow cytometry to determine RBC, WBC, PLT counts, while colorimetric method was used for Hb content determination. All analyses were performed by Siemens ADVIA 120 Hematology System. Statistics on PCBs effects on investigated parameters were performed using ANOVA followed by Fisher's LSD post hoc test in SPSS software and *P*-values less than 0.05 were considered significant. Dose-response relationship has been investigated by PROAST software. **Results and Discussion:** PCBs produced statistically significant changes in WBC and PLT count and dose-response relationship has been confirmed for these parameters. However, no changes in RBC and Hb were observed at all investigated PCBs doses. Total WBC counts were significantly changed by PCBs in the following manner: lower doses of PCBs (0.5-2 mg/kg/day) produced significant increase, while highest dose of PCBs produced significant decrease of WBC count. Effects of PCBs on WBC, i.e. PCBs immunotoxic effects have been shown in previous studies, proving immune system as potential target for PCBs toxicity. However, the results observed in this study revealed the fact that immunological response of rats depends on PCB doses, low doses producing immunoenhancement while high doses inducing immunosupression. Significant decrease in PLT was observed only with the highest dose of PCBs. This finding can be explained by possible PCBs effect on synthesis of thrombopoietin, a protein that mediates PLT production and which is mainly produced in liver, one of the major targets of PCBs toxicity. **Conclusions:** Results obtained in this study indicate that subacute exposure to PCBs may alter hematological parameters and immune response in rats.

Keywords: PCBs, hematology, rats

EX 39- INTERACTIONS BETWEEN CD AND BDE-209 ON BLOOD CELLS COUNT IN RATS - MULTIPLE FACTORIAL REGRESSION ANALYSIS

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Introduction: The mixture of toxic metal cadmium (Cd) and decabrominated diphenyl ether (BDE-209) as a significant representative of polyhalogenated organic compounds can be detected in the environment, especially in electronic waste landfills. Exposure to these high persistent chemicals continuously, mainly via food ingestion. **Objective:** The aim of this study was to investigate interactions between Cd and BDE-209 for the effects on the number of red blood cells (RBC), white blood cells (WBC) and platelets (PLT) in rats. **Materials and Methods:** Cd and/or BDE-209 were given by oral gavage to the male Wistar rats weighing 200–240 g for 28 days. Animals were divided in 17 groups (6–8/group): control group (water), control vehiculum group (dimethysulphoxide - DMSO), groups treated with three different doses of Cd (2.5, 7.5 or 15 mg Cd/kg/day), groups treated with three different doses of BDE-209 (1000, 2000 or 4000 mg BDE-209 /kg/day), and nine groups treated with both Cd and BDE-209 (3X3 experimental design). Blood samples were taken at the end of experiment and hematological parameters were determined using blood cell counter. Multiple factorial regression model was used for statistical analyses using equation: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 +$ ... (Y-predicted value of the measured effects, X-an independent variable corresponding to the scaled doses of Cd or BDE-209 from -1 to the +1, b_0-intercept, b_1 and b_2-the estimated regression coefficients, b_{12}-characteristic of an interaction between examined toxic chemicals when applied as binary mixture). **Results and Discussion:** The obtained results indicate that exposure to Cd or BDE-209 did not induce statistically significant alterations in investigated hematological parameters: RBC, WBC and PLT, neither if given at low nor if given at high doses levels. However, co-exposure to these chemicals produced statistically significant decrease in number of RBC, while WBC and PLT number were increased. Since b_{12} was positive in equation obtained for the effect of the mixture on RBC,synergism was assumed. No interactions, i.e. additivity were observed for the effects of mixture on WBC and PLT number. Application of Cd and BDE-209 mixtures resulted in disorders of investigated haematological parameters that could be explained by their effect on bone marrow and spleen, followed by inflammation. However, precise mechanisms of the effects of Cd and BDE-209, given alone or in mixture, on blood cells are difficult to be postulated and could be result of different direct or indirect effects of these toxic chemicals. **Conclusions:** This study gives one more piece of evidence that a mixture of different chemicals can produce certain toxic effects not observed for single component of mixture.

Keywords: cadmium, BDE-209, mixture, hematology, rats

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 40- ACUTE TOXICITY OF THE PEPTIDE Phα1β ISOLATED FROM *Phoneutria nigriventer* SPIDER IN WISTAR RATS

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Introduction: Phα1β is a peptide purified from the venom of the Brazilian "armed" spider *Phoneutria nigriventer* which blocks high-voltage calcium currents in type N, R,P/Q and L voltage-gated calcium channels (VGCCs). The Phα1β showed similar efficacy intrathecal to ziconotide however the therapeutic index was better than this one in preclinical models of chronic neuropathic and cancer pain.Substances capable of blocking these channels have been explored as novel analgesics. We investigated the effect of Phα1β spider toxin in animal model, producing maximal analgesia with doses in which it did not induce potential side effects. In contrast, the maximal analgesia induced by x-conotoxin MVIIA was only observed in doses that caused severe side effects. **Objective:** To evaluate the intrathecal acute toxicity of the peptide Phα1β isolated from *Phoneutria nigriventer* spider in Wistar rats. **Materials and Methods:** 50 adult Wistar rats obtained from Lutheran University of Brazil (ULBRA-RS) were used with the approval of the Ethics Committee (2014-31P-Canoas). The acute toxicity was recorded in male (n=25) and female (n=25). The rats were allocated in 5 groups (n=5 male and 5 female) treated by intrathecal route with: PBS-10µL (control); MVIIA toxin-200pmol (positive control); Phα1β-200pmol (D1), Phα1β-500pmol (D2) and Phα1β-1000pmo (D3). Male rats were observed for signs of acute toxicity for 6 hours and euthanized 24 hours after. Serum biochemistry and relative organs mass were evaluated. Female rats were observed for sings of acute toxicity during 6 hours, daily for relative body mass and during 14 days for toxicity signs. Female rats were euthanized on day 14. **Results and Discussion:** No significant differences ($p > 0.05$ one-way ANOVA or Kruskal-Wallis) were observed in the relative mass of the organs in males but serum biochemistry revealed significant reduction ($p<0.05$ by one-way ANOVA or Kruskal Wallis) for urea, GOT, GPT and AF to Phα1β and/or positive control groups, however the variations were within physiological limits to species. No significant difference (p>0 05, repeated measures ANOVA) was observed in females relative body mass, but the D2 adrenals was significant smaller than D1 ($p<0.05$, one way ANOVA), nonetheless both values were appropriated for this species. Some clinical signs manifested after treatment show significant differences ($p<0.05$, chi-square): dispnea in males, hyporesponsiveness in females and straub and tremors in both but worse in females. **Conclusion:** The Phα1βpeptide showed a good safety profile assessed in rats, whereas the signs of toxicity were transient and presented in greater doses than those shown analgesic effects.

Acknowledgement: ***CNPq***

EX 41- ACUTE TOXICITY OF ENERGY DRINKS AND ALCOHOL IN WISTAR RATS

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Introduction: Faced with the progressive increase in the consumption of energy drinks, especially associated with alcohol, and the lack of data in the literature about the toxicological impact of the excessive consumption, this study aims to evaluate the acute neurotoxicity induced by energy drink with or without alcohol consumption. **Objectives:** To evaluate the acute toxicity of energy drinks, its constituents (caffeine, taurine and the combination of these), as well as to investigate the influence of alcohol intake associated with energy drink in Wistar rats. **Material and methods:** Male Wistarrats,60 days old, were used (N = 60, approved by CEUA/UFRGS 26689). Animals were treated orally (n = 5/group) with: 10 ml/kg water (Co); three energy drink doses 5 ml/kg (E5), 7,5ml/kg (E7,5), and 10 ml/kg (E10), 3,2mg/kg caffeine (C); 40 mg/kg taurine (T); caffeine + taurine combination (CT); 2 g/kg alcohol(A), energy drink associated with alcohol (E10A), caffeine and alcohol (CA); taurine and alcohol (TA); caffeine and taurine + alcohol (CTA). Clinical signs were observed for 6 hours and after 24 hours, body weight and mortality were observed daily for 14 days (OECD 420), after that, the rodents were euthanized and relative organs weight were evaluated. **Results and discussion:** The ED10 group showed a significant ($p\leq0.0001$, ANOVA for repeated measures/Bonferroni) increase in relative body mass (128.9 ± 1.3%) compared to other groups (between 113.7 ± 2.4% and 119.2 ± 2.2%). There was a significant reduction ($p\leq0,007$, one-way ANOVA/Bonferroni) in relative liver weight of the groups treated with E10A (3.75 ± 0.008%) and CA (3.71 ± 0.002%) compared to other groups (between 3.96 ± 0.12% and 4.26 ± 0.13%). There were significant differences between groups ($p\leq0.01$ chi-square) in relation to clinical signs: Groups E, C, T and CT induce initial reduction of ambulation (30min) with subsequent increase (60min), except the T. In the E10A group, an increase in ambulation and exploitation (60min), was observed. Tachypnea was more intense in groups E (E5, E7,5, E10, E10A), CT, CA, TA and CTA; hypnosis was increased in groups A, TA, CTA and reduced in the E10 group. **Conclusion:** The energy drink and its constituents expressed signs of acute toxicity characterized by increased ambulation, and tachypnea. Besides, the energy drink was able to reduce alcohol-induced hypnosis.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 42- EVALUATION OF CYTOTOXIC POTENTIAL *IN VITRO* OF *HIMANTHUS DRASTICUS* ETHANOLIC EXTRACT

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Introduction: Cancer is a complex disease with a genetic component, characterized by uncontrolled cell growth. Some drugs in use in clinical oncology were obtained from plants, such as vinblastine and vincristine, both obtained from *Catharanthus roseus* (Apocynaceae). *Himatanthus drasticus* (Apocynaceae) is popularly known as Janaguba and ethnopharmacological studies have shown that the population extensively use the latex collected from the trunk for treating cancer. Thereby, the investigation of cytotoxic activity of Janaguba is relevant to discoveries of bioactive compounds for treating cancer. **Objective:** Evaluate the *in vitro* cytotoxic activity of three extracts of different polarities of *H. drasticus* leaves. **Materials and Methods:** The leaves of *H. drasticus* were collected on the Chapada do Araripe plateau, Crato-CE. The leaves were washed, dried and crushed and subjected to cold exhaustive extraction for 72h, yielding three extracts: ethanolic extract previously delipidated with hexane, 100% ethanolic extract and 50% hydroethanolic (v/v) extract. The extracts were weighed and diluted in sterile DMSO, then submitted to cytotoxic colorimetric MTT assay.in four tumour cell lines To test cell selectivity, the active extract was also tested on peripheral blood mononuclear cells (PBMC). We also ascertained the hemolytic potential of the extracts in mouse erythrocytes from the retro orbital plexus (CEUA n° 116/2014). **Results and Discussion:** Values of CI_{50} lower than 30 µg/mL were considered active for MTT assay. Therefore, only 100% ethanolic extract showed activity, presenting CI_{50} (µg/mL) values of 9.79 in HCT-116 (colon); 8.85 in SF-295 (glioblastoma); 10.12 in HL-60 (leukemia) and 13.59 in OVCAR-8 (ovary). The same assay was done in PBMCs, which showed CI_{50}> 50µg/mL, suggesting selectivity of the 100% ethanolic extract against tumor cell lines. This extract did not show hemolytic potential in healthy mouse erythrocytes at any of the concentrations tested ($CE_{50} \geq 250$µg/mL). The popular use of Janaguba milk has caused density reduction to the species and therefore extraction of the compound from other parts of the plant, for example leaves, could reduce this risk. Moreover, latex consumption poses risks to the population who are unaware of the toxic effects that may occur with indiscriminate use. Hence, phytochemical studies and the mechanisms of action of *H. drasticus* leaf 100 % ethanolic extract are relevant, as it may become a safe phytotherapy for the population. **Conclusions:** The ethnolic extract obtained from *H. drasticus* leaves is a promising source of cytotoxic compounds against tumor cell lines.

EX 43- COCAINE AND ANHYDROECGONINE METHYL ESTER (AEME)-INDUCED NEUROTOXICITY: EXTRINSIC AND/OR MITHOCONDRIAL PATHWAY INVOLVEMENT? - PRELIMINAR RESULTS

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Background: Cocaine abuse has become a health issue, mainly its freebased form, *crack*. Heavy cocaine abuse leads to health problems such as psychotic episodes, violent behavior, brain hemorrhage as well as cognitive functions implications that damages not only functions already acquired, but also new memory acquisition and learning. *In vitro* studies show that cocaine and AEME (the crack's main pyrolysis product) exposure may lead to neuronal death by apoptosis, however, the underlying mechanism involved, which could be the reason for the cognition impairment, still not clear. Aim: Identify the main mechanism involved in neuronal death after exposure to cocaine, AEME and the combination of both drugs. Methods: primary hippocampal cell culture were obtained from rats fetus at 18th gestational day and $1.8x10^6$ cell/well were cultured for 7 days at 37^{o}C and 5% of CO_2. Cells were exposed to 2mM cocaine, 1mM AEME and combination of both, for 3 and 6h. Bax, Bcl2, caspase 8, 9 and 3 proteins were quantified by Western-blotting. Densitometry was analyzed by ImageQuant TL 7.1 (GE Healthcare Life Sciences) and the statistic analysis by GraphPad Prim6 software. Results and discussion: Bax and Bcl2 were analyzed at two independent cultures. Caspase (casp) 8 at one culture and, casp9 and casp3 densitometries results were not viable and must to be repeated. Bax expression: 3h of exposition - CA increased in 61% ($p<0.05$); at 6h - COC increased in 60% ($p<0.05$). Comparing 3h and 6h, we observed a decrease in CA Bax expression (90% - $p<0.001$). Bcl2 expression: 3h of exposition - increase of COC (68%), AEME (74%), CA (72%) all three $p<0.05$; at 6h: CA expressed less than AEME (63%, $p<0.05$); CA of 6h of exposition, expressed less then CA of 3h (115%, $p<0.001$). Bax/Bcl2 ratio was only greater at COC (6h) compared to COC (3h - $p<0.01$). Although the prevalence for pro-apoptotic reaction seems to occur at 3h of exposition and the anti-apoptotic at 6h, it is not statistically significant. About casp8, was observed only fragments of it, and not the full length. Generally, it seems that the smaller fragments (34 and 25kDa) had a decrease expression at 3h of all drug exposition and had an increase at 6h, when compared to control cells. At 3h, AEME and CA decreased the 37KDa fragment expression but at 6h the CA was triggered and increased, when compared to control ($p<0.001$), COC ($p<0.01$) and AEME ($p<0.01$) separately. Conclusion: Until now our preliminary results indicate that the apoptotic stimulus occurs after 3h of exposition, but the cell recovers after 6h. Casp8 fragmentation after 6h of exposition could indicate another death stimulus. Even thought the results showed are reliable further experiments need to be performed in order to confirm our observations.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 44- RADIOPROTECTION STUDIES WITH ETHYL PYRUVATE AND ALPHA PHENYL-N-TERT-BUTYLNITRONE (PBN)

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The toxic action of ionizing radiation originates in an activation of cell water molecules leading to the production of free radicals and other reactive species. These species react with cellular molecules (DNA, proteins, lipids, etc.), initiating the processes leading to damage. There are compounds with known radioprotective action but showing significant toxic effects (eg. WR-2721). There is an interest in the development of substances or mixtures of substances that may protect against ionizing radiation acute effects with lower toxicity. In this work, we developed an experimental model using Sprague-Dawley rats (both sexes, eight animals per group) exposed to X-radiation at a dose of 2 Gy (whole body). After 48 hours, animals were sacrificed and histology of six locations of the gastrointestinal tract (duodenum, jejunum, ileum, colon, salivary glands) and testis were performed. In addition, leukocyte and erythrocyte count and leukocyte formula were performed. Genetic damage was evaluated by the Comet assay in blood. Survival curves up to 60 days post-irradiation were also performed. The radioprotective effect of alpha phenyl-n-tert-butylnitrone (PBN) at doses of 20 and 40 mg/kg (i.p. in saline, one hour before irradiation) was tested. Separately, ethyl pyruvate was administered as a single dose of 50 mg/kg (i.p. in saline, one hour before irradiation) followed by repeated administration for one month in the drinking water (0.3% v/v). Control groups with the compound under test were run simultaneously. Histology of irradiated animals showed inflammatory processes in the epithelia of the digestive tract and in the testis, with no changes detected in salivary glands. Leukocyte count was drastically reduced compared to the control values, presenting also an altered formula. The effect of PBN on the tested parameters was moderately protective when it was administered at the highest dose, highlighting the recovery of erythrocytes in males and the protection of the epithelium in the small intestine (both sexes) and in the testis. No statistically significant protection in the recovery of the level of leukocytes or leukocyte count were observed (both sexes). The genetic damage revealed in the irradiated animals was not reversed by the treatment with PBN, neither a protective effect for survival was observed at any dose tested. The effect of ethyl pyruvate administration resulted in a protection of the epithelium in the duodenum (both sexes), a statistically significant increase on the survival of females but no protective effect on blood parameters (48 hours). In conclusion, both compounds showed a moderate radioprotective action, that is suitable to be improved by increasing doses or treatment times, due both are substances with low toxicity by themselves.

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EX 45- *IN UTERO* BETAMETHASONE EXPOSURE CAUSES DELAY IN PUBERTY ONSET AND ALTERS TESTIS MORPHOLOGY IN MALE RATS

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Introduction: Betamethasone is a glucocorticoid of choice for antenatal treatment to promote fetal lung maturation, thus decreasing the incidence of respiratory distress syndrome and neonatal mortality. Previous work reported impaired sperm quality and fertility in adult male rats after prenatal treatment with betamethasone. **Objective:** The aim of this study was to evaluate the male reproductive parameters during the initial sexual development after *in utero* betamethasone exposure. **Materials and Methods:** Pregnant Wistar rats (n=12/group) were treated with vehicle or 0.1 mg/kg betamethasone on gestational days 12, 13, 18 and 19. The following parameters were observed: Maternal weight gain, anogenital distance and male offspring weight at postnatal day (PND) 1, PND 21 (weaning), and after preputial separation (after PND30). At PND 45, a subset of male animals from each mother was killed and body and reproductive organ weights, serum hormone levels (FSH, LH and testosterone), testicular morphology and morphometry were evaluated. **Results and Discussion:** Maternal weight gain was reduced in the betamethasone group ($p \leq 0.05$). It was observed a significant reduction in the weight of male puppies (n=12) in the betamethasone group at PND 1 ($p \leq 0.05$). However, this reduction in body weight was transient, and by PND21, there were no significant differences. There was a delay in puberty onset in the *in utero* betamethasone treated group according to the age of initial and final preputial separation. Beyond that, at PND 45, testosterone levels were decreased as well as seminal vesicle weight, differently of testicular weight, that was increased. Morphometric analysis of testis showed a significant decrease in the diameter of semiferous tubules from treated group and the histological analysis indicated a significant decrease ($p \leq 0.05$) in morphologically normal tubules (97.9% versus 95.6% in the betamethasone group). In the treated group, many tubules present a disruption in germ cell organization and distribution. In these tubules were observed abnormal migration of Sertoli and germ cells towards to the lumen. **Conclusions:** Our results demonstrate that *in utero* exposure to betamethasone alters reproductive parameters during the development of male offspring. Thus, *in utero* betamethasone exposure can impact peri-pubertal development of males and results in testicular damage that can be manifested in adulthood.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 46- NEW INSIGHTS OF INTERACTION (INHIBITION, REACTIVATION AND AGING) OF ORGANOPHOSPHORUS COMPOUNDS WITH HUMAN ACETYL CHOLINESTERASE USING MASS SPECTROMETRY AND QMMM APPROACHES

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Introduction: Organophosphorus compounds (OPs) are a large and diverse class of chemicals currently used mainly as pesticides and chemical weapons. Exposure to OPs can cause several neurotoxic effects. The acute toxicity is produced by the irreversible inactivation of the enzyme acetylcholinesterase (AChE). Inhibited AChE can be reactivated by cleavage of the Ser-phosphorus bond either spontaneously or through a reaction with nucleophilic agents and can also lose part of the molecule by progressive dealkylation over time in a process called aging. Reactivation of the aged enzyme has not yet been demonstrated. **Objective:** Here our goal was to study oxime reactivation and aging reactions of human AChE (hAChE) inhibited by the pesticides paraoxon (POX) and mipafox (MPX) and an analog of the chemical warfare agent sarin (fluorescent methylphosphonate Flu-MP) by peptide fingerprinted mass spectrometry (MS) method using a MALDI-TOF-TOF method. A quantum mechanics/molecular mechanics (QM/MM) model was created in order to study the post-inhibitory interactions of the OPs diisopropylfluorophosphate (DFP), sarin and MPF with hAChE. **Methods, results and discussion:** A peptide fingerprinted MS method, which clearly distinguished the peptide with the active serine (active center peptide – ACP) of the hAChE adducted with OPs, was developed by MALDI-TOF-TOF. The ACP was detected with a diethyl phosphorylated adduct after POX inhibition, and with an isopropylmethyl phosphonylated and a methyl phosphonylated adduct after Flu-MP inhibition and subsequent aging. Nevertheless, nonaged nonreactivated complexes were seen after MPX inhibition and incubation with oximes, where MS data showed an ACP with an *N,N* diisopropyl phosphoryl adduct. The molecular structures of hAChE aged by MPX, DFP and sarin were modeled by applying the Gaussian 03 software, using as template the crystal structure of the aged tabun-inhibited hAChE. Plots of activation energy versus the distance of atom separation during dealkylation in the molecular modeling experiments may indicate that for the eventual aging MPX, would require high activation energy. **Conclusions:** We document here direct evidence for a phosphorylated hAChE by MPX refractory to oxime reactivation, although we observed no aging by kinetics and MS. Computational molecular modeling has shown energetically preferred aging in the cases of OP-AChE conjugates formed by sarin and DFP as opposed to any of those formed by MPX. These structural data are important in the design of new reactivators of OPs pesticides and chemical weapons. Both MS and QM/MM methods developed here would be useful to understand better the toxicological interactions of AChE with other OPs.

EX 47- VIABILITY EVALUATION OF CULTURED FETAL RAT THYROID USING THE MTT COLORIMETRIC ASSAY.

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Introduction: The thyroid gland functional units organize as independent epithelial spheres, called follicles, which are composed of thyrocytes and a few C-cells. In rodents, the thyroid gland is fully developed by gestational day 18 as measured by iodine uptake and response to TSH stimulation. Cell and follicle cultures are commonly used to study the effects of physiological modulators and xenobiotics on the thyroid, however, follicle three-dimensional organization together with the interaction of thyrocytes with surrounding components more closely mimic in vivo conditions. The MTT assay is a simple colorimetric method widely used for determination of mammalian cell survival and proliferation, it has also been applied to small live whole tissues to evaluate their viability. The aim of this study was to apply MTT colorimetric assay to qualitatively evaluate whole organ culture viability. **Materials and Methods:** Two groups of thyroid glands, including adjacent trachea segments, were excised from Sprague-Dawley rat foetuses at gestational day 20.5. Each thyroid was immediately placed on vials containing Hank's medium at 37°C and one group of thyroids was incubated for 2 h while a second group for 24 h. They were then placed on MTT containing Eppendorf tubes and cultivated for 1 h at 37°C. At the end of incubation time colorant was extracted and absorbance was read at 570 nm. A third independent set of thyroids were cultivated at 70°C for 60 min as a positive control of decreased viability. **Results and Discussion:** Mean absorbance of the heat-treated thyroids was significantly lower than those cultured at 37°C by 2 and 24 hours indicating that the assay is suitable for evaluation of viability of cultured fetal thyrocytes. No significant differences were observed between means absorbance of thyroid cultured for 2 and 24 h. This may imply that at 24 h thyroid remains viable under culture and testing conditions. **Conclusions:** Results indicate that the MTT assay is able to differentiate between viable and non-viable whole organ cultures qualitatively. Further functionality tests are required to assess the correlation between this colorimetric assay and the performance of the gland.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 48- CHRONIC EXPOSURE OF METHAMIDOPHOS AND ROUNDUP®AT LOW DOSES CAUSE INFERTILITY IN MALE MICE

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Introduction: Methamidophos and Roundup® are pesticides broadly employed in agriculture, and even with restrictions on its use, cause damage to health. These pesticides are used indiscriminately, which leads to contaminate the environment.Research shows that these xenobiotics act directly in the male reproductive organs, causing infertility. It's intoxication usually occursby eating fresh food,which are marketedwith pesticide residues,in concentrations allowed or not by ANVISA (National Health Surveillance Agency). The male reproductive system is very sensitive to chemicals commonly used in agriculture. Male infertility has become a public health problem, so, this work tries to delimit the extent of the effect caused by low concentrations exposure of these pesticides on the male reproductive system. **Objective:** Evaluate the effects of methamidophos and Roundup® (glyphosate) exposure on fertility of male mice. **Methods:** 120 adult Swiss mice (40 male and 80 nulliparous females), 50 days old, mature and 40g body weight, from the Central Vivarium UFG. The male were divided into four groups: Sham (vivarium control); Control (water via oral/gavage); Methamidophos (via oral/gavage) dose of 0.004mg/KgPC;Glyphosate: Roundup®, via oral/gavage, dose of 0.005mg/KgPC. The mice were treated during60 days. The dose of pesticides followed the minimum values accepted by ANVISA and CONAMA, respectively. After intoxication period, each male mated with two females, of which, one underwent caesarean at 19 days of gestation, and the other gave birth after 21 days of gestation. The following reproductive parameters were analyzed: pregnancy rate, fetal viability rate, sex ratio and masculinity index. **Results and Discussion:** There was no difference between treatments for pregnancy rate. The animals treated with methamidophos showed a decrease of 39% in fetal viability, and 5.3% in the male:female ratio. On the other hand, the males treated with glyphosate showed a decrease of 2.5% in fetal viability. **Conclusion:** The insecticide methamidophos has negative effects on male mice fertility, even if ingested in low doses. This pesticide is able to change the fertility of mice, even at low concentrations, which are considered safe by ANVISA.But the herbicide Roundup®, virtually did not affect the fertility of male mice.

EX 49- EVALUATION OF THE ACUTE TOXICITY AND THE CYTOTOXIC, GENOTOXIC AND ANTIGENOTOXIC POTENTIAL OF THE BACTERIAL CELLULOSE

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Introduction: Bacterial Cellulose (BC), an exopolysaccharide, is a natural product obtained from sugarcane molasses by flotation in the form of the gelatinous matrix, composed of stable polymerized sugars. Chemical composition and physical properties indicate BC seems to be a promising material applied in the biological sciences and in medicine.
Objective: To analyze the acute systemic toxicity, cytotoxicity, genotoxicity and antigenotoxic potential of the BC.
Materials and Methods: BC gel to 0.8% was produced by bacterial synthesis at the Experimental Station of Carpina (UFRPE). BC cytotoxicity was evaluated in C3A hepatoma cells (HepG2/C3A) culture calculated by measurement percentage of lactate dehydrogenase (LDH). The acute toxicity test was performed in adults Wistar rats (10), that received a single dose of BC (2.000mg of BC/Kg bw) by gavage. The clinical signs of toxicity were observed and after sacrifice, the animals were submitted to necropsy for macroscopic examination the organs. For the in vivo mammalian erythrocyte micronucleus assay were included mice adults (50), Swiss Webster, divided into five groups: two negative control, a one positive control group (CP) and two treated groups (BC). Animals were observed once daily for clinical signs of toxicity. Twenty-four hours after the last treatment, all animals were sacrificed to obtain cell suspensions from the femur bone marrow. The ratio of polychromatic erythrocytes (PCE) to the total number of erythrocytes and frequency of micronucleated polychromatic erythrocytes (MNPCE) were calculated. Data were analyzed and was considered statistically significant if $p<0.05$.
Results: There was no alteration in the LDH release in the wells where C3A cells received the BC solutions (0.33-170µg/mL) compared to the wells where the cells received the culture medium only. No clinical signs of toxicity were present in the treatment with BC and the animals gained weight. No abnormality was found after necropsy. No observed clinical signs of toxicity or mortalities by the micronucleus test. BC administration to the animals at dose level of 200 mg/kg bw did not show statistical significant changes in the ratios MNPCE/PCE in either sex as compared to the negative control. The BC attenuated CP-induced effects. BC treatment showed the statistical difference in the ratios PCE/NCE as compared to the positive control group.
Conclusion: BC did not cause adverse effects when using the high single dose. The LDH data release and mutagenicity studies suggest the BC it was not cytotoxic or genotoxic. The concomitant exposure to BC attenuates cytotoxic CP-induced effects.

Keywords: Antigenotoxicity; Bacterial Cellulose; Biomaterial; Cytotoxicity; Genotoxicity.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 50- MATERNAL AND DEVELOPMENTAL TOXICITY OF AYAHUASCA TEA IN WISTAR RATS

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Introduction: Ayahuasca is a psychoactive beverage prepared with the vine of *Banisteriopsis caapi,* which contains ß-carbolines, an inhibitor of monoamine oxidase, enzyme that degrades the neurotransmitter serotonin, and the leaves of *Psychotria viridis*, which contains N,N-dimethyltryptamine (DMT), an agonist of serotonin receptors. The beverage is used in Brazil in the religious rituals of União do Vegetal (UDV) other religious groups. **Objective:** In the present work, maternal and developmental toxicity was evaluated in *Wistar* rats. **Materials and Methods:** Pregnant rats were exposed daily from the 6th to the 20th gestational day at the doses of 1, 2, 4 and 8X. Caesarean sections were performed on day 21 and implantations, living and dead fetuses, and resorptions were recorded. Reproductive toxicity was evaluated according to the OECD 414/2001 protocol. **Results:** At least one animal from each treated group showed piloerection, tremor and lethargy. Eleven animals from the 4X and 13 animals from the 8X group died during the study. Rats from the 8X group had lower uterus weight than controls, and treated rats had a higher number of total and early absorptions and lower number of fetus than controls. Morphological alterations observed in fetus from treated groups were found in liver, ureter and brain, in addition to wrong positioning of testis and ovaries. **Conclusion**: This study showed maternal toxicity and lethality of ayahuasca in pregnant rats exposed for 15 days at doses corresponding to 4 and 8X the usual dose used in a ritual of UDV. Embryotoxicity was observed in all treated groups. This study showed the toxic potential of ayahuasca, however, should note that the use of tea in UDV and other religions usually occurs once every 15 days, and that daily use, abusive, does not occur in the religious context.

EX 51- A CRYPTORCHIDISM-ORCHIDOPEXY MODEL TO STUDY TESTICULAR DAMAGE BY ENVIRONMENTAL CHEMICALS

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Introduction: Cryptorchidism is a male congenital defect and a risk factor for infertility and testicular germ cell tumors (TGCT). Cryptorchidism, hypospadias, infertility, and TGCT are elements that, isolated or combined, compose the Testicular Dysgenesis Syndrome (TDS). The increased incidences of TDS have been ascribed to some environmental endocrine disrupting chemicals. Testicular abnormal location is considered to be responsible for poor semen quality and germ cell tumorigenesis. The surgery that relocates the testis into the scrotum, orchiopexy, should be performed between the 6th–12th months of age to allow normal fertility and reduce the risk of malignancy. **Objectives:** 1) To characterize the testicular changes induced by a rat cryptorchidism/orchiopexy model with the purpose of better understanding TDS. 2) To present improved surgical methods for establishment of abdominal cryptorchidism and of orchiopexy. **Material and Methods:** Male 3-week old Sprague-Dawley rats were submitted to cryptorchidism by anchoring the albuginea, and not the peritesticular fat, to the abdominal wall, what is the critical step of the model. Some rats were euthanized after 3, 6 or 11 weeks to document morphologically the progression of cryptorchidism-induced testicular alterations. Other animals were also submitted to orchiopexy 3, 5 or 9 weeks after establishment of cryptorchidism. Orchidopexy was performed by anchoring the testes, and not the cauda epididymis, into the internal surface of the scrotal wall; these animals were euthanized 3 or 8 weeks later. Animals submitted to sham cryptorchidism and orchiopexy had their abdomens opened and closed at the same moments as the surgical groups. At least 10 and 5 rats were used in each surgical and sham group, respectively. **Results and Discussion:** Cryptorchid testes showed decreased weights, germ cell apoptosis and reduced germinal epithelium, with spermatogenesis disruption; some tubules presented a Sertoli cells-only pattern. All these findings have been already described in other mechanical methods for inducing cryptorchidism, in which the risk of spermatic cord torsion and testicular atrophy jeopardize the experiment. Herein, three and eight weeks after orchiopexy, lower testes weights were still observed, but spermatogenesis recovered progressively and fully. The proposed rat model of cryptorchidism/orchiopexy was successful and may be useful to study TDS-related testicular alterations in cryptorchid rats exposed to xenobiotics. **Conclusion:** In this study, eight weeks of recovery was optimal to restore the germinative epithelium. However, an ideal moment to perform orchiopexy was not determined since testicular recuperation was the same when orchiopexy was induced either at 3, 5, or 9 weeks after cryptorchidism.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 52- THE CO-ADMINISTRATION OF ASCORBIC ACID PREVENTS THE LONG-TERM ADVERSE REPRODUCTIVE EFFECTS PROMOTED BY ROSUVASTATIN ADMINISTRATION TO PREPUBERTAL MALE RATS

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Introduction: Dyslipidemias are occurring earlier in the population due to the increase of obesity and bad eating habits. Statins inhibit the enzyme HMG-CoA reductase, decreasing total cholesterol. Rosuvastatin has pharmacological advantages and higher inhibitory effects when compared to the other statins and delays puberty onset when administered at prepuberty. Ascorbic acid is an antioxidant compound, may increase testosterone levels and has a protective function on male reproductive system. **Objective:** The present study aimed to evaluate whether ascorbic acid administration may reduce or prevent the adverse effects promoted by rosuvastatin administration during prepuberty on male reproductive system. **Materials and Methods:** Male rats were randomly divided into six experimental groups, which received saline solution (vehicle), 3 or 10 mg/Kg/day of rosuvastatin, 150 mg/day of ascorbic acid and 3 or 10 mg/Kg/day of rosuvastatin associated with 150 mg/day of ascorbic acid from postnatal day (PND) 23 until PND 53. The animals were maintained until PND 100, when male rats (n = 10/group) were mated with non-treated female rats to assess their fertility potential. On PND 110, male rats were euthanized and sperm was obtained to evaluate sperm counts, motility and morphology. In addition, reproductive and vital organs were collected at adulthood and assessed in relation to absolut and relative weights. The results were compared among the groups using ANOVA or Kruskall Wallis folllowed by Tukey or Dunn test, respectively, according to the characteristics of each variable, $p \leq 0.05$. **Results and Discussion:** Rosuvastatin administration decreased sperm motility and sperm counts in the testis, increased tail sperm abnormalities and raised post-implantation loss at the higher dose. Ascorbic acid co-administration was effective in preventing these rosuvastation exposure outcomes. Final body weight and organ weights were not altered due to the treatments with rosuvastatin and/or ascorbic acid. **Conclusion:** The co-administration of ascorbic acid prevents some long-term deleterious reproductive outcomes in male rats exposed to rosuvastatin during prepuberty.

EX 53- IN UTERO EXPOSITION TO CADMIUM AND DIMETHOATE (SINGLE OR MIXED) CHANGE FETAL PROGRAMING AND INCREASE ANXIETY IN RATS OFFSPRINGS DURING DEVELOPMENT.

ANSELMO, F.[1]; FARIA, C.A.[1]; YASSUDA M.M.[1]; HORTA, D.F.[1]; SILVA, D.A.F.[1]; KAWASHIMA, J.D.[1]; DE FRAIA, D.[1]; MACHADO, F.D.[1]; CARVALHO, C.C.[1]; GODINHO, A.F.[1]

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Introduction: Exposition to the insecticides and heavy metals, as for example, dimetoato (DM) and cadmium (Cd), substances of high toxicological impact, produces cumulative effect in the human and animal organisms when the continuous exposition exists, being able to culminate with permanent sequels and a diverse and more higher incidence of illnesses, including neurobehavioral effects provoked by toxic action on the central nervous system (CNS). A behavior generally affected by these chemical groups is anxiety. **Objective:** To evaluate if fetal programming for anxiety behavior may be influenced by gestational exposition to cadmium and dimethoate. **Material and methods:** Groups of 15 pregnant females received during pregnancy: Control (Ct) - only water filtrated; Cd - water containing cadmium acetate (10mg of Cd^{++}/liter); DM - water containing dimethoate (40mg/liter); and Cd+DM. During the postnatal development (PN) at young (PND30) and adult (PND80) ages, the neurobehavioral activity of anxiety was evaluated utilizing: 1) Open Field Arena (OF) for evaluate number of crossings by center of apparatus and latency time for crossing it; 2) Elevated Plus Maze (EPM) for evaluate number of entries and permanency time in arms of the apparatus. **Results e discussion:** The number of crossings in the OF center was decreased at PND30 by all treatments in relation to control; in PND80 the number of crossings in the OF center was decreased by DM and Cd+DM treatments. The latency time increased at PND30 by DM and Cd+DM, and at PND80 by Cd+DM treatment. In EPM apparatus, at PND30, the percentage of permanency in closed arms was unchanged by DM or Cd but was increased by Cd+DM treatment; at PND80 a percentage of permanency increased by DM and Cd+Dm treatments, in relation to Ct and Cd. At PND30 the percentage of closed arms entries was higher for Cd+Dm group in relation to Ct; at PND80 the percentage of closed arms entries was higher in relation to Ct, in all treatment groups. The OF, likely the most popular test of emotionality, provides the behavioral measure used as our selection criterion. Under both natural and laboratory conditions, rodents tend to avoid open spaces where they cannot perform thigmotaxic behavior (physical contact with an object). In other hand, a number of entries increased and a higher permanency time in closed arms of EPM, a classical apparatus to assess anxiolitic/anxiogenic behaviors, also reflex increased anxiety, for the same reason. C**onclusion:** The occurrence of anxiety alterations in young age and persistence at adult age suggests negative influence of treatments in the fetal programming, due to *in utero* chemicals exposition. The findings indicate that the mixture of substances here studied provoke enhanced anxiety.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 54- SUBACUTE EMAMECTIN BENZOATE EXPOSURE MODIFIES ANXIETY AND MOTOR COORDINATION IN RATS

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Introduction: Brazil has been the world´s largest consumer of pesticides since 2008, consumption that has not stopped growing. In 2007 Brazilian Health Surveillance agency (ANVISA) denied permission to use the active principle emamectin benzoate due to its neurotoxicity. However, in 2013 the product was allowed in case of a phytosanitary emergency caused by a pest called *Helicoverpa armigera* in some Brazilian states. In vertebrates, toxic effects of emamectin occur via poisoning of the central nervous system (CNS) through reactions at the receptor for the inhibitory neurotransmitter y-aminobutyric acid (GABA). **Objective:** Evaluate the effects of subacute exposure to low doses of emamectin on behavioral parameters for anxiety and motor coordination in rats. **Material and Methods**: forty five male, adults, Wistar rats received orally (gavage), daily, for three days, one of the following treatments: distilled water (as control); emamectin benzoate (emamectin 5 mg/Kg - E5) and emamectin benzoate (emamectin 10 mg/Kg - E10). On the day after after the last administration the rats were assessed for neurobehavioral activity for anxiety, using the elevated plus-maze (EPM – number of entries and time spent in the open and closed arms), the light/dark box test (LDB – time spent in the light compartment, latency to enter the dark compartment, number of transitions and risk-assessment behavior), and for motor coordination, using the hole-board (HB – pawdip and head-dip). The statistical analyses of the results was performed by ANOVA followed by Tukey´s test ($P<0,05$). **Results and Discussion:** EPM and LDB apparatus are instruments largely utilized to evaluate experimentally anxiety. In EPM the animals of E5 and E10 had decreased number of closed arms entries, however only the E5 have a statistical significant difference; E10 group showed a reduction in time spent in the closed arms. In LDB the E5 and E10 group spent more time on the light compartment of the box. The pawdip behavior in HB apparatus is correlated with motor coordination and head-dip with exploration, in rats. In HB animals of E5 and E10 group increased the number of head-dips and pawdips. The toxic effects of emamectin might have occurred by the stimulatory action on GABA neurotransmitters and as GABA is an inhibitory neurotransmitter on central nervous system, wich could explain the anxiolytic effect observed. Additionally, alterations on motor coordination are inversely correlated with GABA release and might indicate an adverse effect of emamectin, and anxiolitic action would interfere in the motor coordination but exploration also interferes. **Conclusion:** Subacute exposure to emamectin benzoate reduced anxiety and provoked motor incoordination in rats.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EX 55- AUTOPHAGIC PROCESS IS ACTIVATED IN HEPG2 CELLS AND MEDIATES BDE-100-INDUCED TOXICITY

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PBDEs (Polybrominated diphenyl ethers) are used as flame retardants in various products. Nowadays, they are already considered as environmental contaminants due to the fact that they are present in high concentrations in human samples, such as blood, adipose tissue and breast milk, and they are also found in animal wildlife. Many studies assessing toxicity have shown induction of cell death, and our group has already shown hepatotoxicity induced by mitochondrial damage. Thus, the main objective of this study is to contribute to understanding the mechanism of liver toxicity of this compound. Briefly, HepG2 cell line was maintained in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin, in 5% of CO_2 at 37 ^{o}C, and treated with three concentrations selected from preliminary toxicity tests that were carried out previously (0.1, 5, and 25 μM) for 24 and 48 hours. After exposure to BDE-100, we performed immunocytochemistry to assess autophagy phenomena; the cells were fixed, permeabilized and incubated overnight with primary antibody (anti-LC3 –Sigma– 1:1000). Then, cells were incubated with secondary antibody conjugated with Alexa-594 (anti rabbit –Life Technologies– 1:2000) and also with Hoechst (1mg/mL – Sigma), and further inspected and imaged using a fluorescence microscopy. In addition, the cells were labeled with 100nM LysoTracker Red DND-99 (for 30 minutes), in order to investigate the distribution of lysosomes in the cells. Thereafter, DNA was isolated using a specialized kit (Qiagen GmbH) and quantified in a Nanodrop instrument (Thermo Scientific); mitochondrial DNA copy number was assessed using qPCR, by the number of DNA copies of a mitochondrial encoded gene (Cytochrome b), normalized against a nuclear encoded gene (Pyruvate kinase). Statistical analysis was performed using ANOVA and Dunnett post-hoc tests. After the exposure to BDE-100, the cells showed an increase in the conversion of LC3, from LC3-I to LC3-II (the specific cleaved form of the protein that integrates the autophagosome membrane), observed through a transition from diffuse to punctual accumulation of LC3. When wortmannin (100 nM) was pre-added, this classic autophagy inhibitor induced a decrease in LC3-punctual pattern, concomitantly with an increase in the nuclear fragmentation (apoptotic feature). Simultaneously, the cells exposed to BDE-100 showed an increased staining with the lysosomal dye, corroborating the effect of this toxic in inducing autophagy. Furthermore, the cells exposed to BDE-100 showed a decreased mitochondrial DNA copy number, both at 24 and 48h, pointing out the idea that cells exposed to this compound are trying to manage mitochondrial damage by selectively degrading mitochondria (damaged mitochondria) by autophagy. Thus, HepG2 cells exposed to selected concentrations showed activation of autophagy as a putative attempt to manage mitochondrial damage and thus maintain cell viability after exposure to BDE-100.

KEY WORDS: BDE-100, autophagy, flame retardant.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 56- *Erythroxylum deciduum* A. St.-Hil. IN PLANARIAN MODEL

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Introduction: *Erythroxylum deciduum* is an important species of the genus *Erythroxylum* occurring in Brazil. There are reports that leaves and fruits are toxic to sheep that consume in summer, while in winter is not toxic. We have detected the consumption by humans and was reported sensation welfare. Planarians have a relatively well-organized central nervous system and represent the most primitive example of centralization and cephalization of the nervous system. Several neurotransmitters such as dopamine were identified allowing neurochemical studies. Dopaminergic stimulation induces typical hyperkinesias, whereas dopaminergic blocking (example sulpiride) decrease motility: similar neurochemical functional mechanisms as in mammals. **Objective:** We evaluate the effects of ethanol extract of *E. deciduum* A. St.-Hil. on locomotion in planarian model. **Materials and methods:** Dried leaves, collected in summer, were washed, in soxhlet apparatus, with nonpolar solvents and after extracted with ethanol, it was dried and stored until time of use. Individual planarians were placed in microtubes per one hour containing water treated with ions (Group 1 and Group 2) or sulpiride 10^{-8}M (Group 3). After, individual planarians were placed into a clear plastic petri dish containing water treated with ions (G1) or extract 0,1% (G2 and G3) and this located over paper with gridlines spaced 0.5 cm apart. The locomotion was quantified as the number cumulative of gridlines planarians crossed in 10 min observation period. **Results and discussion:** The extract (G2) increased the locomotion (402 ± 8,5 gridlines) in relation to the water (G1) (287 ± 4,9 gridlines) being reversed in presence of dopamine antagonist (G3) (268 ± 4,0 gridlines). **Conclusions:** This shows that the *Erythroxylum deciduum*, in summer, produces active metabolites of the dopaminergic system. This may explain, in part, the effects caused in humans and sheep.

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EX 57- XENOBIOTICS LOW CONCENTRATIONS PROMOTE DELETERIOUS EFFECTS IN MICE

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INTRODUCTION: Pesticides are risk factors to human health, and are used by various productive sectors, mainly by agriculture. Due to the need for increased food production farmers use available market pesticides, such as Methamidophos and Glyphosate, in exaggerated and inadequately form. The Methamidophos is an insecticide whose marketing is prohibited. Nevertheless, it is still illegally used. Glyphosate is an herbicide commonly used in agriculture. Research shows that these xenobiotics may act directly in different body organs, causing adverse health effects. Studying intoxicated mice organs, with these pesticides, is necessary to delineate the resulting exposure dimension to these pesticides, and thus better understand its effects. **OBJECTIVE:** Evaluate the effect of Methamidophos and Roundup® (glyphosate) on the organs mice mass. **MATERIALS AND METHODS**: In this experiment was used 40 males mice, Swiss, 50 days old, mature, 40g body weight, from UFG, that were divided into 4 groups: Sham (vivarium control); Control (water via oral/gavage); Methamidophos: via oral/gavage, dose of 0.004mg/KgPC; Glyphosate: Roundup®, via oral/gavage, dose of 0.005mg/KgPC, (acceptable minimum doses by ANVISA and CONAMA, respectively). In the 15th day of treatment was carried out euthanasia for collecting and weighing the organs. **RESULTS AND DISCUSSION:** The oral sub-acute toxicity induced by methamidophos promoted a significant decrease in body weight of the testicles, and the gastrocnemius muscle. On the other hand, glyphosate provided a significant reduction in epididymal and liver weight, and an increase in epididymal adipose tissue weight. **CONCLUSIONS:** Exposure during 15 days to insecticide methamidophos, even at low concentrations, regulated as safe by ANVISA, demonstrated its deleterious effect on body, testicular and muscular weight. Likewise, Roundup® herbicide (glyphosate), at a dose accepted as safe in drinking water by CONAMA, also promoted a reduction in mice liver and gonad weight. These data proves that there isn't a safe dose of such pesticides, even at very low concentrations, causes deleterious effects in the body.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 58- EFFECT OF ACUTE INTOXICATION WITH MANCOZEBE IN SEXUAL BEHAVIOR OF MALE MICE

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Introduction: In the last years, is observed a rise in diseases related to the indiscriminate use of pesticides, causing hormonal, metabolic and reproductive. disorders, Mancozebe is a widely used fungicide, whose toxic effects have been studied in several species. However, at the present time, no studies have evaluated the potential, motivation and sexual performance, that indicates the effect on the male reproductive system, that find a scientific basis to raise awareness of society about the risks of daily consumption of food contaminated with pesticides, targeting the control of this pesticide, searching for scientific basisto raise awareness of society about the risks of daily consumption of food contaminated with pesticides, aiming for the use control of this pesticide. **Objective:** To evaluate the effects of the acceptable daily exposure of mancozeb in sexual behavior of male mice, using as reproductive parameters: (1) number and frequency of mounts, intrusions and ejaculations; (2) copulatory efficiency. **Materials and Methods**: In this experiment was used 30 males mice, Swiss, 50 days old, mature, 40gbody weight, from UFG, that were divided into 3 groups: Sham: no intervention; Control: (water via oral/gavage) and Mancozeb: (via oral/ gavage) dose 0.03 mg / KgPC. The dose of the pesticide was calculated according to the minimum values accepted by ANVISA in food.After 15 days of treatment, each male was placed with a nulliparous female mature (50days old, 30g body weight) in estrus, and sexual behavior was recorded during 30minutes, in a video camera. Then, video analysis was performed to assess reproductive parameters. **Results and Discussion:** The group treated with mancozebshows worse performance than other groups, such as: total number and average frequency of mounts and the total amount of intrusion and ejaculation. This demonstrates that the libido and sexual potency of these animals are affected.It was also observed a low copulatory efficiency ratio, pointing that the effectiveness of sexual consummation is compromised. **Conclusion:** Male mice intoxicated during 15days with mancozeb fungicide (0.03mg/ KgPC) showed changes in sexual behavior by the reduction of sexual motivation.Behaviorslike this complicate the reproduction and contribute to the increase of infertility. The exposure to the fungicidepromotedchanges insexual performance. These data suggests that contamination with this fungicide affect sexual behavior by decreasingsexual motivation, and consequent infertility.

EX 59- CYTOTOXICITY ASSESSMENT OF AQUEOUS EXTRACT OF LEAVES FROM *Anacardium occidentale* L. FRONT NORMAL AND TUMOR CELLLINES.

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The use of medicinal plants has grown significantly and this rises the interest of research. *Anacardium occidentale* L. is a Brazilian native species, known as cashew. It is rich in phenolic compounds, flavonoids and tannins and has various known therapeutic properties as anti-inflammatory and antimicrobial. The search for new compounds with antiproliferative and antioxidant activity has been increasingly necessary. Because of the this species properties, the aim of this study was to evaluate the cytotoxic effect of aqueous extract of leaves from *Anacardium occidentale* L. Concentrations of 0.1; 1.0; 10 and 100 ug/100 ul were used against cell lines 3T3 (mouse fibroblast), HepG2 (human hepatocellular carcinoma), 786-0 (kidney carcinoma) and B16 (melanoma) for 24 h exposure to the extract, by the MTT method. The absorbance of each well was measured at 570nm ELISA reader. 3T3 cells showed cell growth in all concentrations of the extract ranging from 21.04 to 645.70%. 786-0 cells showed low cell growth dose 0.1 to 10 mg/100 ul. However the concentration of 100 ug/100 ul showed 54.44% cell death. HepG2 cell line showed a high cell growth at all doses studied, ranging from 745.56 to 1274.80%. B16 showed cell growth at concentrations of 0.1 to 100 ug/100 ul, ranging from 21.90 to 51.13%. At the concentration of 1000 ug/100 ul the extract promoted 59.86% inhibition of B16 cell line. The *A. occidentale* extract showed cytotoxicity to cells to 786-0 and B16, which indicates the therapeutic potential. So, deeper studies are necessary of the extract.

Keywords: Cytotoxicity, cashew, cell culture.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 60- OXIDATIVE STRESS AND MORPHOLOGICAL CHANGES IN SALIVARY GLANDS OF RATS AFTER BINGE ETHANOL EXPOSURE

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Introduction: Ethanol consumption in episodic and intense pattern (binge) has been common among teenagers and increased in females at higher levels, with a consumption pattern of 3 times a week[1]. However, salivary glands are poorly investigated about these effects, in which chronic consumption during adolescence has been reported as responsible for morphological changes[2]. **Objective:** To investigate morphological and biochemistry effects of binge ethanol consumption in parotid and submandibular salivary glands of rats from adolescence to adulthood. **Materials and Methods:** Wistar female rats (n=40) received ethanol by gavage at 3g/kg/day (20% w/v) for 3 consecutive days/ week between 35-60 days of age (end of adolescence and early adulthood). Animals were divided in four groups: G1, treated with ethanol for 1 week; G2, treated for 4 weeks with ethanol; C1, treated with distilled water similarly to G1; C2, treated with distilled water as G2. In morphological analysis of glandular tissue, immunohistochemistry for smooth muscle actin (α-SMA), cytokeratin 18 (CK-18) and vimentin were conducted and measured with Image J software, regarding pattern of immunoreactivity. Biochemical changes in glandular tissue were analyzed by two oxidative stress parameters: concentration of nitrites and levels of malondialdehyde (MDA), a biomarker of lipid peroxidation. The difference between pattern of immunoreactivity and oxidative stress was evaluated by Mann-Whitney test ($p \leq 0.05$). **Results and Discussion:** Tissue markers evaluated showed less CK-18 expression in parenchyma of parotid gland exposed to ethanol after 1 ($p=0.009$) and 4 weeks ($p=0.023$). There were no differences in CK-18 expressions for submandibular. As for α-SMA expression in mioepithelial cells, there was lower expression in parotid, only in 1 week ($p=0.002$) of exposure to alcohol. Lower α-SMA expression after 1 ($p=0.016$) and 4 weeks ($p=0.004$) were observed for submandibular. There were no changes related to vimentin in observed periods. Oxidative stress analysis showed differences between levels of MDA at 1 week for submandibular and at 1 and 4 weeks for parotid gland. The concentration of nitrite denoted no difference in any of the evaluated periods in both glands. **Conclusions:** Ethanol binge consumption during adolescence promotes tissue and biochemical changes with only one binge in acinar and mioepithelial cell parotid glands. Submandibular glands seemed to be more resistant to the intoxication model proposed. We demonstrated for the first time that the salivary gland structure may suffer biochemical and cytoskeletal changes with only three days of exposure to ethanol during adolescence.

References

1. M. Parada *et al.*, *Alcohol Clin Exp*. **35**, 8 (2011).
2. L.M.P. Fernandes *et al.*, *Histol. Histopathol.* **30** (2015).

EX 61- EVALUATION OF MERCURY DEPOSIT, OXIDATIVE STRESS MARKERS AND CELL VIABILITY IN PAROTID AND SUBMANDIBULAR RAT SALIVARY GLANDS AFTER CHRONIC METHYLMERCURY INTOXICATION

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Introduction: Mercury is an extremely toxic heavy metal with natural emission to the biosphere, which makes it a factor of importance in toxicology. Among the species of mercury, the organomercury compounds are those, which have greater toxicity, with high risk of exposure at low doses for a long period of time. Although many studies show the relationship of the metal with a variety of clinical disorders, little is known about its effect in the salivary glands.
Objective: To analyze the mercury deposit, oxidative stress markers and cell viability in parotid and submandibular rat salivary glands, after chronic methylmercury intoxication.
Materials and Methods: Two groups of fifteen 40-day-old female Wistar rats were used in the experiment. The animals of the first group were intoxicated by intragastric gavage with MeHg at a dose of 0.04 mg/kg/day over 35 days. The second received distilled water for the same period, representing the control group. After intoxication, the animals were sacrificed and glands were collected for measurement of total mercury deposited in the glandular parenchyma by atomic absorption spectrometry. Assessment and quantification of cell viability were performed using the CellTiter-Glo® assay, following the manufacturer's instructions (Promega, G7570). The levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, and nitrites present in the samples were measured. All data were tabulated and statistically analyzed for normality (Shapiro-Wilk test) and for differences between the samples (Mann Whitney test, $p < 0.05$).
Results and Discussion: According to the results of the statistical analyses, we observed mercury deposits in glands of animals exposed to MeHg, with greater deposition in parotid gland, compared to the submandibular gland ($p=0.0159$); smaller number of viable cells in parotid ($p=0.0189$) and submandibular ($p=0.0189$) glands of intoxicated animals; higher nitrite levels in submandibular glands of intoxicated animals ($p=0.0115$), with no difference of these levels in parotid glands. However, the analysis showed higher concentration of MDA in the parotid of mercury group ($p=0.0136$), not occurring differences between submandibular glands of control and intoxicated animals. From this, it is possible to indicate that MeHg was able to accumulate in the salivary glands in the proposed model of intoxication, causing oxidative damages and resulting in decreased cell viability.
Conclusions: Intoxication by MeHg has generated deposits in the salivary glands, with parotid gland being more susceptible to the total mercury accumulation than the submandibular glands. Oxidative stress culminated in a decrease of cell viability in both types of glands evaluated, although occurring through different pathways.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 62- TOXICITY ASSESSMENT OF NOVEL TETRAHYDROQUINOLINE MOLECULES IN ENDOTHELIAL CELLS

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Introduction: One of the main problems in the pharmaceutical industry is the inability to identify adverse effects of new compounds during preclinical and clinical studies. Building *in vitro* data sets that can accurately predict adverse effects *in vivo*, would allow select compounds with the lower risk profile. An *in vitro* toxicity assessment that could meet this goal, it's a multiparameter analysis of various cellular targets, a range of concentrations of a compound and more than an incubation time. The tetrahydroquinoline molecules (THQ), have a wide range of pharmacological activities, one of that has attracted increased interest, is its effect on the a7 nicotinic acetylcholine receptor (a7 nAChR), a new endothelial target for revascularization in therapeutic angiogenesis. Two THQ, TQS and 4BP-TQS behave as modulator and allosteric agonist of a7 nAChR respectively. Although there is no information of its toxicity, it's expected that like other allosteric modulators of a7 nAChR, they don't present toxic effects. Moreover it's known that related compounds, as Salsolinol and 1BnTIQ exhibit neurotoxic activity. **Objective:** To investigate the possible cytotoxic effects of four THQ, THQ-2, THQ-3, THQ-7 and THQ-26, through a multiparametric analysis that considered three biomarkers on human umbilical vein endothelial cells (HUVEC) exposed to a range of incremental concentrations ($10^{-3,5}$ to 10^{-9} M) of the molecules in study and two times (6 and 24 h) of incubation. **Materials and Methods:** HUVEC were isolated from umbilical cords (Jaffe *et al.* 1973). The sulforhodamine B assay is used for cell density determination, based on the measurement of protein total content. The neutral red uptake assay (lysosomal function) and MTS assay (mitochondrial activity) provides a quantitative estimation of the number of viable cells in a culture. The results were expressed as percentage of viability compared to negative control (199 medium with 5% FBS). SDS 0.2% and H_2O_2 0.02% were used as positive controls, and DMSO 0.3% as solvent control. **Results and Discussion:** THQ-3, THQ-7 and THQ-26 don't exhibit statistically significant toxic effects in a concentration and/or time-dependent manner on the biomarkers evaluated in HUVEC. Only THQ-2 ($10^{-3,5}$ M) at 6 h, showed a slight but not significant downward trend in NRU and SRB assays (97.49 ± 6.85% and 91.70 ± 10.36%, respectively (n=3)).The lowest percentages of viability were observed in the MTS assay at 24 hours. **Conclusions:** THQ-2, THQ-3, THQ-7 and THQ-26 don't exhibit statistically significant toxic effects on the biomarkers evaluated in HUVEC. This allows continuing with their assessment on the angiogenesis in HUVEC.

References

Jaffe et al. *J. Clin. Invest.* 52(11):2745-2756.

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EX 63- EVALUATION OF TERATOGENIC EFFECTS OF MISOPROSTOL USING A LATE ORGANOGENESIS RAT EMBRYO CULTURE

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Introduction: Prostaglandins of the E-series play an important role on bone metabolism and limb development. Misoprostol is a prostaglandin E1 analog and is registered in Chile for the prevention and treatment of non-steroidal anti-inflammatory peptic ulcers. It is also used to induce abortions. In utero exposure to misoprostol, after failed abortion attempts, has been associated to joint and skeletal defects, although the mechanism of action which mediates this teratogenicity is unknown. Since misoprostol can act on prostanoid receptors expressed during limb development, we hypothesized that this interaction could explain the induction of limb defects. We used a late organogenesis embryo culture to study misoprostol –induced skeletal defects. **Objective**: To evaluate the teratogenicity of misoprostol using a late organogenesis rat embryo culture. **Materials and Methods:** GD13.5-collected rat fetuses were cultured for 24 hours at 37 °C, in rat serum diluted in Hank´s solution which also contained either 200, 2.000 or 20.000 pg/mL misoprostol, 70 pg/ml PGE2 or 0,1 µM, AH6809, an EP1 and EP2 antagonist. 1000 mg/L sodium penicillin G and 50 µg/ml ketoconazole were used as negative and positive control, respectively. Formamide (FDA) was used as solvent. After culturing, embryos were evaluated for functionality, growth and morphology. Histology was evaluated with routine haematoxilin and eosin and alcian blue and alizarin red for skeletal and cartilage staining. **Results and Discussion:** There were no statistically significant differences in viability or growth of embryos cultured in the presence of penicillin G, ketoconazol, formamide, misoprostol, PGE2 or AH6809 (prostanoid receptor antagonists). However, skeletal defects were overtly induced at all concentrations of misoprostol and were similar to defects observed in PGE2- and AH6809-treated embryos. **Conclusions:** Misoprostol induces overt alterations in limb development, which may not be mediated by prostanoid receptors EP1 or EP2. The fact that misoprostol induced skeletal defects but did not decrease growth or viability makes this system a good model to study misoprostol-induced teratogenicity.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 64- LIVER PRO- AND ANTIOXIDANT EVALUATION OF *UNCARIA TOMENTOSA* TREATMENT IN RATS

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Uncaria tomentosa (UT) (Willd.) DC (*Rubiaceae*) known as "Cat's claw", is a medicinal plant usually commercialized as a phytotherapic drug, employed worldwide for its anti-inflammatory and immunomodulatory properties[1]. UT extracts have also been shown to possess antioxidant properties, playing a protective role against reactive oxygen species and decreasing oxidative stress in inflammatory process and related conditions[2]. Considering this, a dried bark UT extract was administered to healthy rats, and total (GSHT) and oxidized glutathione (GSSG), thiobarbituric acid reactive substances (TBARS) content, as well as antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were assessed in rat liver homogenates to better verify this possible antioxidant activity. For that purpose, 32 male Wistar rats were randomly divided into a control group and 3 UT-treated groups (UTG). Rats from the UTG groups were treated once daily with dried extract of UT suspended in water in the concentrations of 15 (usual therapeutic dose), 75 and 150mg/kg of body weight by gavage for 28 consecutive days. On day 29, all rats were deeply anaesthetized with xylazine and ketamine (5 and 50mg/kg of body weight, respectively). Intravenous perfusion with ice *cold* 0,9% NaCl solution was performed. Then, the liver was collected and processed for GSHT and GSSG[3] and TBARS[4] assays for lipid peroxidation assessment. The activities of antioxidant enzymes were expressed based on the protein content of the samples[5]. Despite the antioxidant properties attributed to UT, our study showed that the subchronic administration of the UT extract to *healthy* rats, by itself, did not alter the pro and antioxidant parameters assessed. However, the antioxidant protection may be elicited by concomitant prooxidative conditions, not tested in this study.

References: 1-K. Keplinger et al., J. Ethnopharmacol. **64**, 23-34 (1999). 2-M.E. Heitzman, Phytochem. **66**, 5-29 (2005). 3-I. Rahman et al., *Nat*. Protoc. **6**, 3159-3165 (2006). 4-C. Fraga et al., Biochem. Pharmacol. **36**, 717-720 (1987). 5-O.H. Lowry et al., JBC. **193**, 265-275 (1951).

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EX 65- REPRODUCTIVE TOXICOLOGICAL EVALUATION OF AYAHUASCA IN MALE RATS

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Introduction: Ayahuasca is a psychoactive plant infusion used in rituals by native communities in the Amazon and by Christian religious groups. It is prepared from *Psychotria viridis* bush leaves which contain DMT, an agonist of serotonin receptors, and *Banisteriopsis* caapi vine, which contains β-carbolines, MAO inhibitors. Some studies demonstrate the relationship between psychoactive drugs and male infertility. However, no toxicological study has ever been conducted to verify the toxicity of ayahuasca to male fertility. **Objective**: To evaluate the toxicological potential of ayahuasca in the fertility of male rats. **Materials and Methods:** Sixty 6-week old male Wistar rats of uniform weight (205±10g) were randomly divided into 5 groups (N=12). Treated groups received ayahuasca every other day for 70 days at 1X, 2X, 4X and 8X the usual dose used in a religious ritual (100 mL/70 kg bw) and the control group received filtered water. The animals were observed daily for clinical signs of toxicity with animal weight and feed intake recorded every three days. At the end of the treatment, all animals were euthanized by CO_2 exposure. Necropsy was conducted, followed by organ collection and weighing. Blood was collected by cardiac puncture for biochemical and hormone (testosterone, LH and FSH) analysis. D*uctus deferens* contents were extracted and added to 1 mL of pre-warmed (34° C) DEMEM culture medium for sperm motility analysis. A testis and epididymis of each animal were processed to obtain total sperm count, daily sperm production and sperm transit time. Counts were performed in a Neubauer chamber using an optical microscope under 400X magnitude. The other testicle was fixed in *Bouin* for histopathological analysis. Sperm morphological analysis was conducted by perforating the remaining epididymis and extracting cells for slide confection. **Results and discussion**: Two animals from the 8X group died minutes after administration, one of them due to gavage failure. A significant decrease in feed consumption and decrease in total weight gain of animals from the 4X ($p = 0.04$) and 8X ($p = 0.01$) groups compared to controls were observed. The relative stomach weight was significantly higher in animals treated with the 8X dose compared to controls. Preliminary hormonal assay results showed a significant increase in total testosterone of animals from the 4X group compared to controls. Analyses of other reproductive indicators are ongoing. **Conclusion**: The decrease in feed intake and total weight gain of the treated animals is dose-dependent (4X). Animals treated with this dose had higher testosterone levels than the control group. Reproductive indicators, mobility and sperm morphology results, together with testicle histology, will be presented and discussed.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 66- BEHAVIOURAL ASSESSMENT OF MALE WISTAR RATS TREATED WITH CHRONIC DOSES OF AYAHUASCA

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Introduction: Ayahuasca is a psychoactive plant infusion used in spiritual rituals by native Amazon communities and Christian religious groups, especially in Brazil. It is a concoction prepared from *Psychotria viridis* leaves, which contain dimethyltryptophan (DMT), an agonist of serotonin receptors, and the vine of Banisteriopsis caapi that contains β-carbolines, which act as MAO inhibitors. Previous work conducted by our research group has shown that ayahuasca has a potential antidepressant action in rats acutely exposed at high doses,[1] an effect that should be further investigated. **Objective:** To investigate the potential effects of chronic ayahuasca in male Wistar rats. **Materials and Methods:** Seventy-two 6-week old male Wistar rats (210 ± 10g) were randomly divided into 6 groups (N = 12). Treated groups received ayahuasca by gavage at 1X, 2X, 4X and 8X the usual dose used during a religious ritual (100 mL/70 kg bw) every other day for 70 days. The negative control group received filtered water and the positive control was treated with fluoxetine (10 mg/kg). Behavioural tests (open-field, elevated plus maze, and forced swimming tests) were conducted 1 hour after the first gavage; after 36 days of treatment (middle of treatment); and 70 days of treatment. **Results and discussion:** In the open field, significant decrease was observed in the number of central quadrant entries for the 2X and 8X treated groups and in the rearing for the 2X, 4X, and 8X treated groups during the 36 days of evaluation compared to positive control. In the forced swimming test, there was a significant decrease in swimming for groups 1X, 2X and 8X, and increased immobility for those treated with 1X the dose following 36 days of treatment when compared to the positive control. In the elevated plus maze, animals treated with the 1X dose spent significantly less time in the closed arms during the first behavioral evaluation compared to the positive control. No significant differences were observed between the ayahuasca treated groups and the negative control. **Conclusion:** Male Wistar rates treated with chronic doses of ayahuasca at 1 to 8X administered every other day did not exhibit anxiolytic nor antidepressant behaviour. These results indicate that the intermittent regime was not sufficient to maintain the antidepressant effect of ayahuasca seen previously.

1. Pic-Taylor et al**.** *Behavioural Processes*, 18, 102-110, 2015.

EX 67- GENOTOXIC EVALUATION OF THE ETHANOLIC EXTRACT OF *BAUHINIA FORFICATA* LINK (FABACEAE) IN HEPG2 CELLS

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Introduction: *Bauhinia forficata* Link (Fabaceae), known as "pata-de-vaca", is a South America's native species, traditionally used for diabetes mellitus and dyslipidemia treatments, besides other organic disturbs. Regardless of the widespread popular use of this plant species, the therapeutic potential of *B. forficata* has been poorly scientifically explored, notably focusing on the antidiabetic property, with gaps about the safety of its use as a medicine.[1] **Objective**: The aim of this study was to assess the genotoxic potential of the ethanolic extract (EE) of *B. forficata* leaves using the comet assay. **Methodology**: HepG2 cells were maintained under standard culture conditions and treated with EE of *B. forficata* with three different concentrations (25, 125 and 250 µg/mL) for 3 hours. After that, the alkaline version of the comet assay was performed.[2] DNA damage was scored in visual analysis, and the nucleoids were classified (classes 0, 1, 2, 3, or 4) according to the size of the comet tail. The damage index was obtained by the formula, damage index = (0 x n0) + (1 x n1) + (2 x n2) + (3 x n3) + (4 x n4), where the variables n0 – n4 represent the number of nucleoids with 0 – 4 damage level. **Results and Discussion:** After the treatments, cell viability was higher than 90%. The results showed an increase in DNA damage directly related with the increase of the EE tested concentrations. For the negative control and for treatment with 25, 125 and 250 µg/mL of EE were observed predominance of nucleoid classes 0 and 1, 2 and 3, and 3 and 4, respectively. Also, the treatments with 125 and 250 µg/mL of EE resulted in a statistically significant increase in the damage index compared to the negative control. Other studies evaluated the toxicity of *B. forficata* leaves by enzymatic biomarkers in mice, and did not observe clear signs of adverse effects; however, they did not evaluate its genotoxicity.[3] Additionally, in accordance with our study, species of *Bauhinia*, as *Bauhinia platypetala* and *Bauhinia monandra*, demonstrated genotoxicity in different experimental models.[4,5] **Conclusion:** The EE obtained from the leaves of *B. forficata* significantly induced genetic damage to HepG2 cells evaluated by comet assay at concentrations of 125 and 250 µg/mL, suggesting the need to perform more genotoxicity tests to verify the safety of its use in humans as remedy.

References:

1. E.T. Miyake *et al*., *Rev. Bras. Farmacogn*. **1**, 58 (1986).
2. R.R. Tice *et al*., *Environ Mol Mutagen*. **35**, 206 (2000).
3. M. T. Pepato *et al*., *BMC Complement Altern Med*. **4**, 1 (2004).
4. F. J. Santos *et al*., *J Ethnopharmacol*. **144**, 474 (2012).
5. M. F. S. Macêdo *et al*., *Rev. Bras. Farmacogn.* **18**, 509 (2008).

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 68- VARENICLINE INCREASE GENERAL ACTIVITY OF RATS IN THE OPEN FIELD

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Introduction: Varenicline is a synthetic chemical used for the treatment of smoking. Its mechanism of action is by binding to nicotinic cholinergic receptors as a partial agonist of the receptors α4β2 and α3β4 and a full agonist of the receptor α7. These chemical may cause some unwanted side effects, such as nausea, headache, vomiting, cardiovascular and neuropsychiatric effects. Considering that there is a tendency to expand the clinical use of varenicline and that are few studies related to behavioral, cognitive and motor effects, become necessary more studies on their pharmacological effects. **Objective**: The aim of this study is to evaluate the behavior of male rats exposed to a single dose of varenicline in the open field for 7 days. **Material and methods**: Forty male rats received a single dose of varenicline or tap water by gavage: control group (A) - 1mL/kg of water; group B - 0.03 mg/kg of varenicline; group C – 0.1 mg/kg of varenicline; group D – 0.3 mg/kg of varenicline. n=10 male rats/group. Varenicline was obtained from the commercial product Champix® - Pfizer, diluted on tap water. It was evaluated the general activity of these animals in the open field 30 minutes, 1, 2, 4, 6, 24, 48, 72 hours and 7 days after the administration of the drug or water. The parameters evaluated were: frequency of locomotion, rearing, grooming and defecation, and time of grooming and immobility. **Results and discussion**: The analysis of the behavior of the male rats in the open field showed alterations on the following parameters, when compared to the control group: the rats from group D had an increase of the locomotion frequency 6 hours after the administration of the chemical ($p<0.05$). The rats from the group C had an increase of the rearing frequency 30 minutes after the administration of varenicline ($p<0.05$). The rats from the group C had a decrease of the time of immobility 2 ($p<0.05$), 4 ($p<0.05$) and 6 ($p<0.01$) hours after the administration of the chemical and the rats of group D had an increase of the time of immobility 4 ($p<0.01$) and 6 ($p<0.0001$) hours after the administration of varenicline. The rats from group D had an increase of frequency of defecation 48 hours after the administration of the chemical ($p<0.05$). Regard the frequency and the time of grooming parameters, the two-way ANOVA did not showed significant statistical differences between the groups. Thus it was possible to note that varenicline was able to increase the general activity of the rats and the highest and intermediate doses, make them more alert and awake. **Conclusion:** The results indicate that exposure to the highest and intermediate dose of varenicline promoted an increase of the general activity of the rats in the open field.

EX 69- IVERMECTIN REDUCE MOTOR COORDINATION AND LEVELS OF CENTRAL NEUROTRANSMITTERS IN RATS

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Introduction: Ivermectin (IVM) is an antiparasitic of the avermectin group of widely used in Veterinary Medicine for combating parasites. In mammals, a variety of evidence indicates that these chemicals interact with channels chloride mediated gamma-aminobutyric acid (GABA) neurotransmission and this system interferes with motor coordination. **Aim:** Evaluate whether exposure to IVM can interfere with motor coordination and levels of neurotransmitters in the striatum and hypothalamus of rats. **Material and methods:** Male Wistar rats were divided into three equal groups. The experimental groups received 0.2 (therapeutic dose) or 1.0 mg/kg IVM; the control group received 1.0 ml/kg of saline (0.9% NaCl). It was evaluated the motor coordination after 15 minutes and 24 hours of IVM or saline administration. The hypothalamic and striatal neurotransmitters (serotonin – 5HT, dopamine – DA, GABA) and respective metabolites (5-hydroxyindoleacetic acid – 5HIAA, 3,4-dihydroxyphenylacetic acid – DOPAC) were evaluated 24 hours after IVM administration, by high-pressure liquid chromatography (HPLC). **Results and Discussion:** The results showed decreased in motor coordination at 15 minutes and 24 hours after IVM administration in both doses in relation to control group. Regarding the assessment of the levels of neurotransmitters and metabolites in the hypothalamus, reduction was observed in the levels of 5HT at dose of 0.2 mg/kg IVM and at both doses in 5HIAA and GABA levels. In the striatum, reduction was observed in the levels of 5HT and DOPAC in both doses and 5HIAA and GABA at the lowest dose. **Conclusion:** In conclusion, IVM administration in rats promoted impaired motor coordination male rats and this effect can be attributed to IVM action on serotonergic, dopaminergic and GABAergic systems of striatum and hypothalamus.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 70- ASCORBIC ACID PARTIALLY PREVENTS THE DELAY OF PUBERT ONSET PROMOTED BY ROSUVASTATIN EXPOSURE TO PREPUBERTAL MALE RATS

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Introduction: Dyslipidemias are occurring earlier in the population due to the increase of obesity, bad eating habits and sedentary lifestyle. Statins inhibit the enzyme HMG-CoA reductase, decreasing total cholesterol, mainly LDL-cholesterol. Rosuvastatin is one of the last generation statin and shows pharmacological advantages and higher inhibitory effects when compared to the others. Ascorbic acid acts as an antioxidant substance, may increase serum testosterone concentrations and has a protective function on male reproductive system. **Objectives:** The present study aimed to evaluate whether ascorbic acid administration may reduce or prevent the adverse effects promoted by rosuvastatin administration during prepuberty on male reproductive system. **Materials and Methods:** Juvenile male rats were randomly divided into four experimental groups (n = 20/group), which received saline solution (vehicle), 3 mg/Kg/day of rosuvastatin, 150 mg/day of ascorbic acid and 3 mg/Kg/day of rosuvastatin associated with 150 mg/day of ascorbic acid from post-natal day (PND) 23 until PND 53. The age of prepucial separation was considered as an indicative of puberty onset and was assessed since PND 30. Male rats (n = 10/group) were euthanized on PND 53, when reproductive and vital organs were collected and weighed. The remaining animals (n = 10/group) were maintained until PND 100 and were submitted to sexual behavior test. The results were compared among the groups using ANOVA or Kruskall Wallis folllowed by Tukey or Dunns test, respectively, according to the characteristics of each variable, $p \leq 0.05$. **Results and Discussion:** Rosuvastatin-treated rats showed a significant delay of puberty onset, but ascorbic acid administration was capable to prevent partially this delayed puberty onset. The co-administration of ascorbic acid and rosuvastatin showed increased number of ejaculations during sexual behavior test when compared to the group only exposed to rosuvastatin, although this increased number of ejaculations was similar when compared to the control group. **Conclusion:** Ascorbic acid supplementation partially prevents the delay in the age of puberty onset caused by prepubertal exposure to rosuvastatin and may increase the ejaculations of the co-exposed animals during sexual behavior test when compared to the group only exposed to rosuvastatin.

EX 71- PRENATAL BETAMETHASONE EXPOSURE ALTERS INITIAL SEXUAL DEVELOPMENT AND REPRODUCTIVE PARAMETERS IN ADULT FEMALE RATS

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Introduction: Betamethasone (BM) is a potent drug of anti-rheumatic, anti-inflammatory and immunosuppressive action, and it is the drug of choice for antenatal treatment, promoting fetal lung maturation and thus decreasing the incidence of respiratory distress syndrome and neonatal mortality and morbidity. Studies have shown, in rats, that prenatal exposure to this drug promoted changes in testosterone levels and sperm parameters in adulthood, though there are few studies on the female offspring. **Objectives:** To study the possible changes in the age of puberty onset and other reproductive parameters of female rats, with emphasis on the early sexual development, caused by *in utero* exposure to BM. **Material and Methods:** Pregnant Wistar rats were divided (n=13/group) in control group and treated with 0.1 mg/kg of BM on gestational days (GD) 12, 13, 18 and 19. The female offspring were evaluated for: body weight at postnatal day (PND) 1 and anogenital distance (AGD), areolas/nipples count and age of vaginal opening and first estrous (indicators of puberty onset) from PND 30 onwards. Starting on PND 60 and for 15 days estrous cyclicity was evaluated. One female per litter was killed in estrous after PND 75 and reproductive organ weights were determined. Another female was used to perform sexual behavior evaluation and fertility test. Statistics: Student's t-test or Mann-Whitney test, $p<0.05$. (Ethics Committee Protocol 451). **Results and Discussion:** There was a decrease in weight gain of pregnant females after the beginning of treatment, and a decrease of litter weight on PND 1. The exposure *in utero* to BM promoted morphological virilization in females, as demonstrated by the increased relative AGD, which is naturally higher in males. These females also showed an increase in the number of nipples/areolas, delay in the age of puberty onset, as there was delay in vaginal opening and first estrous, and increase in the ovary and uterus relative weights. In this same group the number of estrous decreased and the length of the cycle, in days, increased. The females prenatally exposed to BM performed less lordosis when paired with non-treated males during the sexual behavior test. Intrauterine BM exposure also decreased fertility, as revealed by the increase in post-implantation loss and incidence of reabsorptions, and lower fetal weight registered on GD 20. **Conclusion:** Our results show that prenatal BM exposure impaired sexual development and provoked long-term deleterious effects on morpho-functional reproductive aspects of female rats. Knowing that these parameters are hormone-sensitive, it is possible that these results are due to, at least in part, the influence of the drug on the levels of sex hormones, which is under investigation.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 72- BEHAVIORAL EFFECTS OF SUBCUTANEOUS ADMINISTRATION OF ETHANOL IN SWISS MICE PUPS ON DPN 10

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Introduction: the post natal day (PND) 10 is a critical stage for brain development, comprised in the brain growth spurt (BGS) phase, characterized by periods of especially large increases of brain. In that stage, the neuronal connections are being established and chemical injuries can be toxic. Among the toxic substances the ethanol is known to have an extensive apoptotic neurodegeneration effects throughout many regions of the developing brain. Thus, this can offer new approaches to explore the function of brain regions and their relationship with the animal behavior. **Objective:** to evaluate the behavioral effects of subcutaneous (s.c.) administration of ethanol at the PND 10 in swiss mice. **Material and Methods:** on the DPN 10, fourteen mice pups were separated in two groups. One group was injured with administration s.c. of ethanol (3.5 g/kg) and another group received saline solution s.c. (NaCl 0.9%). After two weeks the animals were submitted to open field test (30 min/session, blocked in three-intervals of 10 minutes) and after the same animals were evaluated in the elevated plus maze (5 min/session). All experiments were performed in accordance with the guidelines of FMVZ-USP (protocol nº 4844200115). **Results and discussion:** in the open field was observed significant increase in jump frequency at the interval 10-20 minutes ($p<0.0139$) versus the control group. Furthermore, we observed that ethanol group showed a reduction ($p<0.0001$) of time (seconds) spent in the central zone, in all interval times analyzed. In the elevated plus maze the ethanol group showed decrease in head dipping frequency ($p<0.0115$) versus control group. In open field results we observed another parameter denominated jump that could be associated with an atypical behavior in ethanol group. Considering the results found in the open field test and elevated plus maze, the ethanol group showed an anxiogenic behavior, spending less time in central zone and decreasing the head dipping frequency, respectively. **Conclusion:** these preliminary results showed that ethanol in the DPN 10 was able to promote a dysfunction behavior. Furthermore, new researches are necessarily to understand how ethanol can act in BGS and behavior.

Keywords: Brain growth spurt, ethanol, mice, open field, elevated plus maze.

EX 73- CHRONIC AND ACUTE TOXICITY OF AYAHUASCA TEA (B. caapi and Psychotria viridis), FOR HISTOLOGICAL ANALYSIS IN RATS WISTAR

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Introduction: Ayahuasca is a psychoactive concoction of two plants: *Banisteripsis Caapi*, rich in β-carbolines and *Psycotria viridis*, containing an indole alkaloid- N, N-dimethyltryptamine (DMT), which is structurally similar to serotonin. It is traditionally used by indigenous Amazonian populations, and since the 1930s, in the rituals of some religious groups in Brazil (Santo Daime, Barquinha,UDV). The ritualistic use is considered legal. Over the last decade, the non-ritualistic use of ayahuasca has led to a series intoxication incidents. **Objectives:** This study evaluated the toxicity of the ayahuasca concoction by visceral and brain histology of Wistar female rats treated orally with single dose and chronically. **Materials and Methods:** Spleen, liver and kidney histological samples were analyzed following acute treatment at 30X and 50X the UDV ritual dose (6 animals/ dose; OECD 423/2001 protocol), and chronic treatment from the 6th to 20th gestational days at doses of 1X, 2X, 4X and 8X (25 animals/dose; OECD 414/2001 protocol). Additionally, the neurotoxicity of pregnant ayahuasca treated rats was evaluated by Nissl staining, an indicator of neuronal viability. **Results and discussion:** The ayahuasca letal dose (LD50) was estimated to be over 50X the ritual dose. Twenty four pregnant rats from the 4X and 8X groups died during the experiment after showing clinical signs of toxicity, including tremors, piloerection, hind limb abduction, cyanoses and convulsion. Significant macroscopic changes were observed in the stomach and intestine of the surviving animals from all study groups, after single or repeated dosing. Histological analysis of acute treatment showed no significant changes compared to the control group. However, histological analysis following chronic treatment showed statistically significant changes in the kidneys of animals treated at the two highest doses. Neuronal quantification also revealed statistically significant cell loss in CA1,CA2, CA3 and raphe nuclei areas, compared to the control group, mainly in the highest doses, with a direct correlation between neural loss and dose. **Conclusion:** Our results indicate that the ayahuasca has a low acute toxicity to female rats, and that daily exposure at the ritualistic dose is safe. However, it is clear that chronic use at doses equal or higher than 4X the ritual dose can harm the kidneys and lead to CNS damage, an indication that the recreacional use of ayahuasca may represent a health risk to humans.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 74- CYTOTOXICITY ASSESSMENT OF AQUEOUS EXTRACT *Schinus terebinthifolius* RADDI FRONT LINES OF NORMAL AND TUMOR CELLS.

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Belonging to the family *Anacardiacea*, *Schinus terebinthifolius* Raddi is a plant native to South America popularly known as red mastic and used in folk medicine as an astringent, antidiarrheal, anti-inflammatory, antiseptic, antioxidant, among other uses. Studies have shown that the medicinal properties of *S. terebinthifolius* are related to the high content of phenolic derivatives such as gallic acid, tannins, flavonoids, which are responsible for their antioxidant and antitumor action for acting in the modulation of genomic repair. Therefore, the aim of this study was to evaluate the cytotoxicity of aqueous extract of the barks of *Schinus terebinthifolius* Raddi in normal and tumor cell lines. Concentrations of 0.1; 1.0; 10 and 100 ug/100ul were used against cell lines 3T3 (mouse fibroblast), HepG2 (human hepatocellular carcinoma), 786-0 (kidney carcinoma) and B16 (melanoma) for 24 h exposure to the extract, by the MTT method. The absorbance of each well was measured at 570nm ELISA reader. The cell 3T3 cells showed 53.37% inhibition only at the concentration of 100 ug/100ul. 786-0 showed significant cellular inhibition at concentrations of 1.0 to 10 ug/100ul of 44.81% and 47.25% respectively. The Hep-G2 cells showed enhanced cell growth at all concentrations, ranging from 1034.6 to 1229.4%%. Against B16 cells the extract showed low cell inhibition, ranging 17.86% only in the concentration of 100 ug/100ul. The cell lines 3T3 and 786-0 were sensitive to this plant extract, highlighting the extract cytotoxicity for these strains. Despite its promising ability as a therapeutic agent, more studies are needed to confirm this findings.

Keywords: Cytotoxicity, aroeira, cell culture.

EX 75- ENVIRONMENTAL TOBACCO SMOKE EXPOSURE DURING THE EARLY POSTNATAL PERIOD DISTURBS SYNAPTIC PROTEINS IN DISTINCT BRAIN REGIONS.

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Background: The Central Nervous System (CNS) development represents a critical period, marked by intense synaptogenesis and synaptic plasticity. Previous study from our group showed that environmental tobacco smoke (ETS) exposure during early brain development induces impairment in cognitive functions. However, little is known about the effects of ETS in synaptic transmission. **Aims**: Evaluate the effects of ETS exposure in the early brain development through the proteins involved in synaptic transmission, such as synaptophysin, synaptotagmin, PSD-95 and EGR-1. **Materials and Methods:** C57/BL mice were exposed to 3R4F cigarette smoke (0.8 mg of nicotine/cigarette) from 3rd (P3) until 14th (P14) day of life, for two hours/day. The animals (n=6) were euthanized at P15 (childhood), P35 (adolescence) and P65 (adulthood). Synaptophysin, synaptotagmin, EGR-1 and PSD-95, were quantified in the hippocampus, cerebellum, striatum and brainsteim by *Western-blotting*. **Results/Discussion:** In hippocampus, the exposure to ETS in the early postnatal period induced a decrease in PSD-95 levels ($p<0.05$) and an increase in synaptotagmin ($p<0.01$) and in synaptophysin ($p<0.05$) levels during infancy. Moreover, there was a decrease in synaptotagmin levels ($p<0.05$) during adolescence. In brainstem, the animals exposed to ETS showed an increase in PSD-95 levels ($p<0.05$) during infancy, a decrease in EGR-1 levels ($p<0.05$) during adolescence and an increase in EGR-1 levels ($p<0.01$) during adulthood. In cerebellum, the mice exposed to ETS showed a decrease in synaptophysin ($p<0.05$) and in EGR-1 ($p<0.05$) levels during adolescence and an increase in synaptophysin ($p<0.05$) and EGR-1 ($p<0.05$) levels during adulthood. In striatum, the exposure to ETS in the early postnatal period induced a decrease in synaptotagmin leves ($p<0.05$) during adulthood. **Conclusion:** The exposure to ETS in the early postnatal period disturbs synaptic proteins in distinct brain regions and some effects observed were not reversed in adolescence and adulthood.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 76- CYTOTOXICITY ASSESSMENT OF AQUEOUS EXTRACT OF FRUIT FROM *Libidibia ferrea* FRONT NORMAL AND TUMOR CELL LINES.

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The *Libidibia ferrea Martius*, popularly known as Jucá, has antifungal, anti-microbial and anti-inflammatory properties. Most studies with the crude extract of Jucá are focused on their analgesic activity, anti-inflammatory, anti-ulcer, cancer treatment and chemopreventive properties. The crude extract of Jucá contains anthraquinones, alkaloids, depsides, depsidones, flavonoids, lactones, saponins, sugars, tannins, sesquiterpenes and triterpenes. Tannins being considered as the main component. Therefore, the aim of this study is to assess the cytotoxic effect of aqueous extract of the fruits from *Libidibia ferrea.* Concentrations of 0.1; 1.0; 10 and 100 ug / 100 ul were used against cell lines 3T3 (mouse fibroblast), HepG2 (human hepatocellular carcinoma), 786-0 (kidney carcinoma) and B16 (melanoma) for 24 h exposure to the extract, by the MTT method. The absorbance of each well was measured at 570nm ELISA reader. The extract showed no cytotoxicity to 3T3 cells at concentrations of 0.1 and 1.0 ug/100ul. However, the concentration 10 to 1000 mg/100ul caused 3T3 cell inhibition ranging from 3.70 to 41.26%. HepG2 cells demonstrated cell growth at all concentrations tested, with growth ranging from 89.47 to 1409.60%, the dose of 100 ug/100ul demonstrated the greater proliferative power. The 786-0 cells showed concentration-dependent cell death, ranging from 11.79 to 56%. B16 showed cytotoxicity at concentrations 100 and 1000 ug/100ul of 30.12 and 67.45%, respectively. The extract *Libidibia ferrea* showed no cytotoxicity in normal cells (3T3) and demonstrated good inhibitory activity to tumor cells 786-0 and B16. Despite the sharp growth in tumor cells HepG2, the extract of the fruits of *Libidibia ferrea* proved quite promising for research into new medicines based on medicinal plants for the treatment of certain cancers.

Keywords: Citotoxicity, juca, cell culture.

EX 77- DIAZEPAM REDUCES TOXICITY IN MICROGLIA UNDER INFLAMMATORY CONDITIONS

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Introduction: The translocator protein (18 kDa) (TSPO) is a benzodiazepine receptor located in the outer mitochondrial membrane. It can be found both in central nervous system and periphery. TSPO expression may constitute a biomarker of brain inflammation and reactive gliosis that could be monitored by using TSPO ligands. However, recent evidence suggests that TSPO may be more than just a biomarker of active brain disease as it is possible that it mediates functional roles in the context of cell activation and inflammation. In fact, TSPO ligands might be valuable in the treatment of neurological and psychiatric disorders associated with neuroinflammation. **Objective:** To investigate the effect of diazepam, a TSPO ligand, in BV2 cells (murine microglia) under basal or inflammotoxic conditions. **Methods:** Murine BV2 microglial cells were seeded in culture plates, exposed to different concentrations of an inflammatory stimuli (LPS, 10 or 100 ng/mL), and co-incubated or not with diazepam (10, 100 or 1000 ng/mL) for 4 or 12 hours. TSPO and CD200R expression was evaluated by FACS, and cytokines (IL1β and TGF) secreted in the culture media were measured by ELISA. Control cells were incubated with cultured media (RPMI with 10% calf serum). **Results and Discussion:** Incubation of BV2 cells with LPS increased TSPO expression (70% vs control), while diazepam *per se* did not. Moreover, diazepam increased the secretion of IL-1β after 4 or 12 hours of treatment (64,34 or 67,80%) and increased TGF secretion after 4 hours of treatment (1000 ng; 57,43%). Surprisingly, co-incubation with diazepam (10, 100 or 1000 ng/mL) inhibited the LPS-induced (10 ng/mL) secretion of IL-1β after 4 hours (60%) of treatment, and it also inhibited reduction of TGF secretion evoked by LPS after 4 hours of co-incubation (10 or 100 ng/mL). We observed the same pattern for the membrane markers. LPS increased CD200R expression (a M2 marker) and this effect was avoid by co-incubation with diazepam (10, 100 or 1000 ng/mL) after 4 hours. **Conclusion:** Incubation with LPS stimulates TSPO expression in BV2 cells and this effect seems to be modulated by diazepam. In addition, diazepam inhibited LPS-induced polarization of BV2 cells to M1 inflammatory profile, leading the cells to a M2 profile. The role of TSPO in this last effect will be further investigated.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 78- CYTOTOXIC ACTIVITY OF HYDROETHANOLIC EXTRACT OF THE LEAVES FROM *Kalanchoe brasiliensis*.

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The *Kalanchoe brasiliensis*, known as saião, is a species rich in flavonoids quercetin and kaempferol, being widely used for the treatment of inflammation, cough, gastritis, among other diseases. Because of this, this species has awakened interest to search for new herbal and bioactive compounds. The objective of this study is to assess the cytotoxic effect of hydroethanolic extract of the leaves from *Kalanchoe brasiliensis*. Concentrations of 0.1; 1.0; 10 and 100 ug / 100 ul were used against cell lines 3T3 (mouse fibroblast), HepG2 (human hepatocellular carcinoma), 786-0 (kidney carcinoma) and B16 (melanoma) for 24 h exposure to the extract, by the MTT method. The absorbance of each well was measured at 570nm ELISA reader. The evaluation of the extract at 3T3 cell line showed cell growth within 24 hours in most of the concentrations, reaching 57.09%. 786-0 showed cell death at all concentrations, ranging from 14.75 to 94.41%, showing the concentration-dependent effect and the concentration of 100 ug/100ul with higher effect. HepG2 cells demonstrated cell growth at all concentrations, with a concentration of 0.1 ug/100ul with 995.80%, and a concentration of 1000 ug/100ul with 771.50% cell growth. The B16 cells showed cell growth with concentration-dependent effect, and cell viability ranged from 28.95 to 73.55%, in concentrations from 0.1 to 1000 ug/100ul. The extract showed inhibition of cell activity primarily to tumor cells 786-0, highlighting its potential antitumor in analyzed circumstances.

Keyswords: *Kalanchoe brasiliensis,* citotoxicity, toxicity.

EX 79- ASSESSMENT CYTOTOXICITY OF EXTRACT OF *Ziziphus joazeiro* IN THE NORMAL AND TUMOR CELL LINES.

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The *Ziziphus joazeiro (Rhamnaceae*), popularly known as "juá", is one of the endemic species of the savanna biome and is widely used in folk medicine as an expectorant in the treatment of bronchitis and gastric ulcers. Displays analgesic, anti-inflammatory, antibacterial, febrifuge and healing and pharmacological activities. Given this, the objective of this study was to evaluate the cytotoxic effect of aqueous extract of *Ziziphus joazeiro*. Concentrations of 0.1; 1.0; 10 and 100 ug / 100 ul were used against cell lines 3T3 (mouse fibroblast), HepG2 (human hepatocellular carcinoma), 786-0 (kidney carcinoma) and B16 (melanoma) for 24 h exposure to the extract, by the MTT method. The absorbance of each well was measured at 570nm ELISA reader. The 3T3 cells showed cell growth at concentrations of 0.1 to 10 ug/100 ul, ranging from 58.53 to 80.33%, while at a concentration of 100 ug/100 ul cell death demonstrated 88.83%. HepG2 cells demonstrated high cell growth at concentrations of 0.1 to 10 ug/100 ul, ranging from 930.37 to 1096.30%, since the concentration of 100 ug/100 ul cell death demonstrated 56.07%. The 786-0 cells showed cell growth at a concentration of 10 ug/100 ul of 38.53%, but the concentration of 100 ug/100 ul showed pronounced cell death 91.11%. The extract showed cytotoxicity in a concentration of 100 ug/100 ul in B16 cell line, with 92.88% cell death. The extract from *Ziziphus joazeiro* showed proliferative power to the cell lines 3T3 and Hep-G2, and cell inhibiting activity for lines 786-0 and B16 at a concentration of 100 ug/100 ul. Only the concentration of 100 ug / 100 ul showed cytotoxicity in all cell lines. So it is interesting to deepen the studies in this direction.

Keywords: Cytotoxicity, cell culture, juazeiro.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 80- EFFECTS OF SUB-CHRONIC EXPOSURE TO RESIDUAL OIL FLY ASH (ROFA) AND RESVERATROL TREATMENT ON RAT LUNGS

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Introduction: Residual oil fly ash (ROFA) is a common pollutant in areas where there is oil burning. As the particle matter (PM), ROFA contains particles from various diameters that can be inhaled by humans and cause damage mainly in the respiratory system. The resveratrol (RVS), a natural polyphenol, has received increasing attention due its varied bioactivities, including the inhibition of tumorgenesis, lipid modification and antiapoptotic action. **Aim:** Investigate the sub-chronic exposure to ROFA and the effects of RSV intake on rat lungs. **Methods:** Thirty-three male Wistar rats were used and distributed into the following groups: control (n=9, CTL), resveratrol (n=8, RSV), residual oil fly ash (n=8, ROFA) and ROFA treated with RVS (n=8, ROFA + RSV). Rats received ROFA suspension (20 µg/10 µl) by intranasal instillation and RVS solution (20mg/kg) by gavage during 14 weeks. After twenty-four hours of the last exposure, rats were euthanized and the lungs were collected to determinate oxidative damage markers (thiobarbituric acid reactive substances - TBARS), antioxidant status (superoxide dismutase - SOD and catalase – CAT activity) DNA damage, metal levels and interleukins (IL-6, IL-1β and TNF-α). This project was approved by CEUA/UFCSPA (n.13/109). All analyses were performed using SPSS software, version 17.0. The significance level was set 5%. **Results and Discussion:** Animals exposed to ROFA showed higher levels of TNF-α (P= 0.047). The group exposed to ROFA and RSV presented higher CAT activity (P= 0.041). There was no difference in stress oxidative and inflammatory markers. Further, there was no difference DNA damage measured by tail DNA % (P= 0.347), tail moment (P= 0.256) and olive tail moment (P= 0.236) and at metals levels (P> 0.005). Studies have shown the exposure to different particles cause oxidative stress and inflammation as well as metals deposition in acute and short-term exposure. To our knowledge, this is the first study that evaluated the effects on lung after sub-chronic exposure to ROFA. Lungs have efficient clearance mechanisms and high adaptive capacity suggesting that the long exposure period could induce an adaptive response and possibly metals have accumulated on extrapulmonary organs. **Conclusion:** Although ROFA induced inflammation, the lungs presented an adaptive response, which avoided oxidative stress and DNA damages.

Acknowledgement: Capes

EX 81- RECREATIONAL USE OF MARIJUANA DURING PREGNANCY IS ASSOCIATED WITH ADVERSE GESTATIONAL OUTCOMES AND IMPAIRED REFLEX AND DECREASED MUSCULAR STRENGHT IN NEONATES

BENEVENUTO S.G.M.[1,2]; BELOTTI, L.[1]; DOMENICO M.D.[1]; COSTA N.S.X.[1]; LOURENÇO, D.T.[1]; RIBEIRO JÚNIOR[1], G; MIGLINO, M.A[2]; SALDIVA P.H.N.[1]; DOLHNIKOFF, M.[1]; VERAS M.M.[1,2]

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Introduction: Prevalence of use of marijuana during pregnancy ranges from 3-30% and with the legalization of recreational use, the percentage may increase. Studies in humans are scarce and indicate that this exposure may impair neurological development of the conceptus; however, many factors (e.g. tobacco and other drugs concomitant use, social and economic status and nutritional factors) could have confounded the results. Besides, existing experimental studies use non-realistic exposure routes and dose. Thus, in order to evaluate the effects of recreational use of marijuana during pregnancy we developed a mouse model of realistic exposure that mimic the use in humans under the aspects of dose and route of exposure. **Aim**: Evaluate the effects of marijuana (*Cannabis sativa*) use via inhalation (low dose) during pregnancy on reproductive, gestational and neonatal outcomes. **Methods:** Pregnant mice (n=20) were exposed (nose-only) daily during 5 minutes to marijuana smoke [0.2g of *Cannabis*] (Group CA) or filtered air (group FA) from 5.5 dpc to 17.5 dpc. Food intake and maternal weight gain were recorded. Pregnancy progression and fetal development were assessed by ultrasound. On 18.5dpc half of the dams were euthanized for fetal and placental macroscopic evaluation. Other half gave birth for gestational length and postnatal development assessment using behavioral tests (hind limb suspension, negative geotaxis and righting reflex). **Results and Discussion:** The number of live fetuses were reduced (CA=7.5, FA=11; $p<0.04$) and an increase in the secondary sex ratio was observed in CA. Food intake was not different, however maternal body weight gain during pregnancy were significantly reduced. Number of pre and post implantational failures was also increased (but not significant). Placental weight and gestational length were not altered, however fetal weight is reduced (less 8% in CA, $p<0.01$) and fetal size is diminished. Evaluation of pregnancy progress by USG indicates that there is a restriction in fetal growth in CA. Neonatal behavioral tests indicate that CA pups present muscle weakness and become fatigued faster than FA; Although CA pups were faster to complete the negative geotaxis test, the frequency of failures were higher in CA compared to FA. The righting test has shown again that CA pups are slower and not always successful in this test compared to FA pups ($p=0.02$). **Conclusion:** We have shown for the first time that maternal exposure to Cannabis in real world conditions of use and dose impairs gestation, compromising fetal development. Neonates of smoking mothers present decreased muscle strength and impaired neonatal reflexes and motor function that could have lifelong consequences.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 82- HEXABROMINATED FLAME RETARDANT (BDE-154) INDUCES APOPTOSIS ON HEPG2 CELLS

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INTRODUCTION- Hexa-polybrominated diphenyl ether is one of the compounds belonging to the class of brominated flame retardant with wide use in daily life and with toxic evidence to several exposed organisms. Nevertheless, reports about its toxicity to human health are limited and need further studies which will help to clarify the risk to human health. **OBJETIVE:** The aim of the work was to investigate the cytotoxic effects of BDE-154 on human hepatoma cells (HepG2) after exposure of 24 and 48 hours. **METHODOLOGY:** After incubation with BDE-154 in concentrations ranging from 0.5 to 25µM for 24 and 48 hours, the following parameters were evaluated: cell proliferation; cell viability; Mitochondrial Membrane Potential; phosphatidylserine exposure, LDH release and activation of caspases 3 and 9. **RESULT AND DISCUSSION:** Our assays demonstrated that exposure to BDE 154 caused several interferences on cell homeostasis. The cell proliferation assay revealed significant impact on cell growth after exposure to high BDE 154 concentrations, these interferences agreed with results obtained by the MTT assay, which evidenced diminished normal mitochondrial cell function at higher concentrations. The evaluation of the mitochondrial membrane potential confirmed the injuries that the 25 µM of BDE-154 caused since the mitochondrial membrane potential was decreased at both evaluated times (24 and 48 h). Cell viability decreased with apoptotic cell death markers (positive Annexin/negative PI). This assay provided further evidence of apoptotic cell death at high BDE-154 concentrations which can be confirmed by decrease of pro-caspase 3 on high concentrations and by the lower mitochondrial cytochrome *c* levels verified in both evaluated periods, indicating that mitochondria pathway was associated with the apoptotic death induced by BDE 154. In addition, the mitochondrial apoptosis cell death also can be confirmed by decreases of pro-caspase 9 retrieved after BDE-154 exposure. **CONCLUSION:** Ours results shows that BDE 154 can cause toxicity to liver cells (HepG2) by apoptosis and the mitochondrial pathways participates in the mechanisms that lead to cell death. These data contribute to evidences of damages about the indiscriminated use of BDE flame retardant and reinforce the need for further research on the toxicity of this retardant class.

Financial support:
São Paulo Research Foundation – FAPESP 2012/06464-6 and 2009/06912-6.

EX 83- EVALUATION OF THE TOXICITY OF HERBICIDES TRIFLURALIN AND TEBUTHIURON ON ISOLATED RAT LIVER MITOCHONDRIA

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Introduction: Due to the expansion of agricultural activities throughout the world, great amount of herbicides has being used in recent years. They act as a chemical control to prevent or destroy weeds, able to attack whole plantations. Among the herbicides which can be employed are trifluralin and tebuthiuron. The first, widely used in soybean fields, belongs to the class of dinitroaniline and acts preventing weed cell division and hence their growth. The second, used in sugarcane plantations, is an inhibitor of photosynthesis process and belongs to the class of substituted ureas. **Objective:** In order to understand the effects of such herbicides in organisms non-target, this work aims to check the effects on oxidative status and bioenergetics of isolated mitochondria, obtained from rat liver. It was chosen this model as toxicological test due to importance for the functioning of the mitochondria in the body, may thus provide important insights into mechanisms of action and toxicity of these xenobiotics. **Materials and Methods:** For this purpose, mitochondria from rat liver were isolated and tested with standard solutions of trifluralin and tebutiurom. **Results and Discussion:** The results showed that tebuthiuron did not alter the values of lipoperoxidation and swelling mitochondrial and neither does trifluralin to lipoperoxidation. However trifluralin was able to cause mitochondrial swelling, situation that was reversed in presence of ruthenium-red, a potent Ca^{+2} channels inhibitor. This phenomenon is caused by the calcium outflow from the interior of the mitochondria, generated by the opening of the permeability transition pore, which causes the osmotic swelling of the organelle. **Conclusion:** The negative results for lipoperoxidation for both herbicides indicate that they are not able of generating reactive oxygen species, but other supporting tests are still needed. In addition, the positive result for mitochondrial swelling indicates that trifluralin can affect Ca^{+2} channels and change the permeability of the mitochondrial inner membrane.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 84- ACUTE TOXICITY OF GLYPHOSATE-BASED HERBICIDE AND CELL VIABILITY IN ZEBRAFISH BRAIN

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Introduction: Glyphosate (*N*-[phosphonomethyl]-glycine) (GLY), and GLY-based herbicides (GBH), are the most commonly used herbicides worldwide. The GBH are complex chemical formulations containing GLY, surfactants and other substances not specified. GLY is classified as an organophosphate and these are recognized by their toxic effects on the central nervous system (CNS). However, few studies have investigated whether GLY, or GBH, can lead to neurotoxicity and also how the CNS cells respond to this herbicide. In this study we use the *Danio rerio,* commonly called zebrafish, to study the possible neurotoxic effects induced by pure GLY and GBH. **Objectives:** Investigate the acute toxicity levels of GBH in this species through determination of the letal concentration media in 96 hours (LC50,96h). Evaluate and compare the toxic effects of different concentrations of pure GLY and GBH on cell viability in *ex vivo* zebrafish brain. Carry out testing *in vivo* chronic toxicity with sublethal concentrations used in different works for standardization studies of neurotoxicity mechanisms using zebrafish brain. **Materials and methods:** For LC50,96h tests, the fishes were exposed to GBH (Monsanto do Brasil Ltda, containing 720g/Kg acid equivalent to GLY), and the mortality was recorded every 24h. For cell viability assay in *ex vivo* zebrafish brain, the brains were dissected and incubated for 3h at 27ºC in different concentrations of GLY and GBH (0, 0.00038, 0.006, 0.06, 10, 25 and 50 mM). After the incubation period, MTT (3-(4,5-dimethylthiazol-2il)-2,5-diphenyltetrazolium) was added (30') and after was added dimethylsulfoxide (DMSO). To testing *in vivo* with sublethal concentrations (0, 0.065, 1 and 10 mg/L of GBH), the brains were dissected and incubated in MTT followed by DMSO, as described above, after exposure period (168h and 360h). The reading was performed using a spectrophotometer and the results were expressed as optical density x 1000/mg tissue. **Results and discussion:** The LC50,96h value was 48 mg/L of GBH. We found no data in the literature to the LC50,96h of GBH in zebrafish for comparison. We found that pure GLY is more toxic that GBH, since they have shown a significant reduction on MTT at 10 mM and GBH only after 25 mM (One-way ANOVA; 60% of reduction; $P< 0,01$). Some studies in the literature indicate that GBH has a higher toxic potential than pure GLY, but our data in an *ex vivo* model showed greater toxicity for pure GLY. We found no significant differences in cell viability after chronic treatment *in vivo*. **Conclusion:** In summary, our findings demonstrate that pure GLY was toxic to the brain as well as GBH in *ex vivo* studies and suggest that this species can be an interesting model to evaluate the involved mechanisms on neurotoxicity induced by GBH *in vivo*.

EX 85- EVALUATION OF THE PHARMACOKINETIC AND ALLOMETRIC PROFILE OF ANTINEOPLASTIC PROTOTYPE DRUG LQFM018 IN EXPERIMENTAL MODELS BY LC-MS/MS

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Introduction: LQFM018 is a candidate to prototype antitumor drug obtained by molecular simplification from anticancer compounds called nutlins, inhibitors of the p53-MDM2 interaction. **Goal:** This study aimed to develop and validate a bioanalytical method for quantification of LQFM018 in plasma and study its pharmacokinetic. **Materials and Methods:** Validation contemplated the following tests: linearity, precision, accuracy, selectivity, recovery, matrix effect, residual effect and stability. The validated bioanalytical method used the following analytical parameters: ACE® C18 column (100 mm × 4.6 mm, 5 µm); mobile phase: buffer 2 mM ammonium acetate with 0.025% formic acid and methanol (50%:50% v/v); flow: 1.2 mL/min; internal standard (IS): domperidone; liquid-liquid extraction with methyl tert-butyl ether (MTBE) and injection volume of 3.0 µL. LQFM018 was administered to 3 females Wistar rats at 100 mg/kg, i.p. After administration, 0.5 mL samples of blood were collected by cannulation of the left jugular vein with heparinized syringe, at 1h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h and 9 h. Blood samples were identified and centrifuged to obtain plasma which was frozen at -20 °C. **Results and Discussion:** The method was linear from 10 to 15,000 ng/mL, intrarun precision was ranged from 0.6% to 5.5%, interrun from 1.8% to 6.7%, accuracy from 99.0% to 107.0% and recovery of 74.1% ± 4.9%. Retention times were 3.16 min (LQFM018) and 1.81 min (IS). Pharmacokinetic parameters (mean ± SD) were: $t_{½}$ = 2.89 h ± 2.0; Cl_T/F = 22.01 mL/min/kg ± 13.5; Vd/F = 5.48 L/kg ± 3.6. To Vd value, applying the allometric scaling for an adult weighing 70 kg, a value of 1954.3 L/kg is found, which suggests extravascular accumulation of the compound, with high distribution, due to the lipophilic nature of the molecule (LogP = 2.48). In allometric scaling, for an individual weighing 70 kg, the value of half-life correspond to 12.6 h. This value may be regarded as a median to large value. Very short half-lives have the disadvantage of repeated administrations per day, whereas the ones with very long half-lives may accumulate in the body and cause toxicity. Total clearance value found resulting in a value of 1800.4 mL/min for a person weighing 70 kg. Comparing this value with the creatinine clearance (120 mL/min), it is possible to realize that the value found is higher, indicating greater participation of hepatic elimination mechanisms once the fact that the fat-soluble molecule of the compound requires a prior biotransformation to increase its polarity so that it can be eliminated. **Conclusion:** Results, extrapolated to humans, revealed large half-life, high Vd and high CL_T, allowing the comprehension that the prototype showed good tissue distribution profile and was extensively eliminated.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 86- STANDARDIZATION OF ENZYMATIC ACTIVITY OF GLUTATHIONE-S-TRASFERASE AND CATALASE IN *Poecilia reticulate*

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Introduction: The standardization of enzymatic assays for kinetic studies is a fundamental step to evaluate enzyme activity. The standardization of enzymes in full speed reaction (Vmax) makes it possible comparisons between biological models on different exposure conditions.The glutathione-s-transferase (GST), and catalase (CAT) are important parameters for toxicological responses and they have specific structural arrangements for each organisms. **Aim:** The aim was standardize the assays of enzymatic activity of GST and CAT in liver of *P. reticulata*. **Materials and Methods:** Livers of *P. reticulata* were weighed and homogenized in potassium phosphate buffer (0.1M, pH 7.0; 0.25M sucrose) at a ratio of 1:8. The protein determination was conducted by the Lowry method with the standard curve establishment from bovine albumin. The GST activity was determined by the formation of conjugated product (GS-DNB) from the reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB). CAT activity was determined by decomposition reaction of hydrogen peroxide (H_2O_2) associated with decrease of absorbance. The absorbance readings was taken at 240 nm for H_2O_2 and at 340 nm for GS-DNB. The standardization was made by variations of pH and concentration of substrates. For CAT the pH values were 5.0- 7.0 and H_2O_2 concentrations were 15-100 mM. For GST pH values were 5.0- 6.5, CDNB concentrations were 0.125-3.0 mM, GSH concentrations were 0.5-5.0 mM. It calculated the Michaelis-Menten constant (KM) and Vmax to define the substrate concentration responsible for great enzymatic. **Results and Discussion:** For the reaction of GST with CDNB, the Vmax obtained was 1.255 $U.mg^{-1}$ and KM =3.125 mM and with the substrate GSH was 0.8051 $U.mg^{-1}$ and KM = 1.688 mM. The saturation of GST and KM values obtained for GSH and CDNB were multiplied by five. The optimum pH for GST was 6.00. To the reaction CAT Vmax obtained with H_2O_2 was 1.083 $U.mg^{-1}$ and KM = 53.92 mM. The value of saturation CAT obtained for the concentration of H_2O_2 was 269.6 mM. The optimum pH for CAT was 5.83. It was observed that pH, when not standardized, results directly alter because the change in absorbance. For each organism, enzymes have small variations significantly influence this value at its optimum. The class Pisces is very diverse biochemical aspects, so it is necessary to carry out standardization studies of enzymatic activity for each species used as biomonitor to establish better conditions for enzymatic activity assays. **Conclusion:** In assays for the enzymatic activity of GST in *P. reticulata* the optimum is obtained with a concentration of 15.625 mM CDNB and 8.34 mM GSH, the tests being carried out at pH 6.00. For the CAT enzyme condition is optimal with a concentration of 269.6 mM H_2O_2 and pH = 5.83.

keywords: antioxidant enzymes, Liver, Michaelis-Menten constant, Pisces

EX 87- GENOTOXIC ASSESSMENT IN THREE TISSUES OF *PIARACTUS MESOPOTAMICUS* AFTER AN ACUTE EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE

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In order to evaluate the effects of herbicides and other contaminants on the aquatic environments, fish are the most used as sentinels, mainly due to their bioaccumulation potential and sensitivity to these chemicals. Even though glyphosate-based herbicides are widely used in South America, few information is available about the toxicity and genotoxic effects to native freshwater fishes. In the current study, the genotoxic effects of the herbicide formulation Roundup Full II® were evaluated in blood, gill and liver cells of *Piaractus mesopotamicus*, species of great ecological and commercial value for the region. The juveniles of *P. mesopotamicus* were obtained from three hatcheries in Misiones (Argentina) and acclimated at least 15 days in 75 litters aquaria in aerated and dechlorinated water conditions. Previously, the lethal concentration 50 (LC_{50}) for 96 hours of exposure was determined, with a theoretical value of 8.92 mg/L. The genotoxic analysis in cells of the three tissues was performed by the comet, micronuclei (MN) and nuclear abnormalities (NA) assays after 96 h exposing fish to a sub-lethal concentration of Roundup Full II® (2.75 mg/L). Simultaneously, a negative control (NC) with specimens exposed to detoxification conditions and a positive control (PC) with fish exposed 96 h to a solution of 15 mg/L of ethylmethanesulphonate (EMS) were performed. The three tissues showed an increased damage index for the comet assay and high frequencies of MN and NA after the Roundup Full II® treatment compared to their respective NC. The gill cells were the most sensitives with the statistically highest MN and NA frequencies. For the comet assay, all tissues appear to have a similar response after the herbicide exposure. When such results are compared with the observations for MN and NA, it should be noted that these assays detect different aspects of genotoxicity because the micronucleus test detects lesions that survive at least one mitotic cycle, while the comet assay identifies repairable DNA lesions or alkali labile sites. Therefore, the amount of repairable and fixed DNA damage detected in each tissue could be related to particular characteristics like their mitotic index, anatomic location, function and the antioxidant status. In conclusion, it can be said that the glyphosate based herbicide Roundup Full II® has genotoxic effects on erythrocytes, hepatocytes and gill cells of *P. mesopotamicus*. These results reveal the presence of diverse kinds of DNA damage, like clastogenic events and the influence of several tissues properties in the amount of reversible and irreversible DNA damage.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 88- HISTOPATHOLOGIC CHANGES IN PAROTID GLAND PARENCHYMA AFTER CHRONIC EXPOSURE TO ALCOHOL IN WISTAR RATS

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Background: Alcohol intake use and abuse is a serious public health problem. Some studies have demonstrated that continuous exposure to ethanol is responsible for alterations in glandular tissue. However, there are few studies investigating the effects of ethanol on salivary gland tissue. **Objective:** The aim of this study was to investigate if chronic ethanol intake is able to induce histopathological changes in rat parotid gland parenchyma. **Materials and Methods**: A total of 15 male Wistar rats were distributed into 2 groups: experimental group (n=10), in which rats were treated with 20% ethanol in drinking water for 7 weeks consecutively *ad libitum* and a control group, non-treated animals (n=5). Histopatological and morphometric analysis were performed by using hematoxylin and eosin stain sections. **Results and discussion**: High ethanol-preferring rats showed alterations in the parenchyma of parotid gland characterized by degeneration of acinar cells and ductal proliferation. Morphometric analysis of acinus area did not show remarkable changes between groups. **Conclusion**: These results suggest that ethanol induces histopathological changes in rat parotid chronically exposed to ethanol.

Keywords: Parotid gland, alcohol, salivary gland.

EX 89- SEIZURES INDUCED BY THE *NERIUM OLEANDER* ETHANOLIC EXTRACT

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Introduction: *Nerium oleander* is a toxic plant used in gardens across the world. It has been described a highly intoxicating effect by leaves ingestion in human, either by accident or suicide attempt. Thus, in order to understand the toxic mechanisms and possible develop better treatment approach to those intoxicated we sought to describe the electrophysiological changes induced by the ethanolic extract injection in rats. **Objective:** Study the eletroencephalografic changes associated with ethanolic extract *Nerium oleander* (EENO). **Materials and Methods:** We collected the leaves in Belém – PA and the ethanolic extract was obtained in the Laboratory of Pharmacology and Toxicology of Natural Products. Under stereotaxic surgery, were implanted circular-tip on the motor cortex of Wistar rats (n=10) coordinate Bregma -1AP, 2 LL. In the 7th day post-op the animals were connected to a data-acquisition system (Amplifier, Grass Technologies P511; Digital acquisition, National Instruments P6112) sampling rate of 1kHz and the data was hard-drive storage for offline analyzes. All analyzes were carried out in a custom-made tool using Python language programming. We recorded a basal period of 10 min followed by i.p. administration of 50 mg/Kg of the ethanolic extract and recorded for 60 min. **Results and Discussion:** The administration of the ethanolic extract was able to induce seizure-like electrophysiological behavior. With increased amplitude of oscillations and cyclic potentials bursts. **Conclusion:** To our knowledge, this is the first report of such brain alteration induced by the *Nerium oleander* intoxication regarding the electrophysiological function of the brain. Moreover it seems to be a suitable convulsant model for development of anti-seizures drugs. Further studies are underway to reveal the best compound to control the seizures induced by this plant and to reveal its seizure-underlying mechanism.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 90- HEMATOLOGIC EVALUATION OF THE OFFSPRING OF MERCURY-INTOXICATED RATS.

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Introduction: Methylmercury (MgHe) is a highly toxic compound, which present accumulation in the food chain. Thus, fish consumption leads to a high exposure to this compound. Offspring contamination during the pregnancy and breastfeeding can have deleterious and toxic effects. **Objective:** Evaluate hematologic effects of MgHe intoxication in the offspring during the pregnancy last third and the breastfeeding period. **Materials and Methods:** Methylmercury (Sigma CA, USA). Animals: Pregnant rats (n=4) received water containing HeMg (0.04 mg/mL) during the last third of the pregnancy and the breastfeeding period. The control grup (n=4) recieved water only. The offspring was divided in two groups: Test Group (n=9) and control grup (n=9). Blood samples were collected from the offspring at on age of 21 days. **Results and Discussion**: The exposed animals presented reduced contage of leukocytes (due to a lymphopenia), erythrocytes, hemoglobina and platelets. **Conclusion**: The results suggests that prenatal and perinatal exposition to HeMg may lead to anemia and coagulation disorders.

EX 91- HEPATOTOXICITY EVALUATION ETHANOLIC BARK EXTRACT OF *HIMATANTHUS SUCUUBA*

LOPES M. S. P.[1], NASCIMENTO L.N.S.[1], CORDEIRO P.G.A.[1], COSTA E.B.R.[1], LOBATO A.M.V.[1], BATISTA L.S.[1] SILVA M. C. F.[2], SOUSA A.S.C.A.[2] , HAMOY M.[1], JÓIA-MELLO. V.[1]

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Introduction: *Himatanthus sucuuba* of Apocynaceae family is a medicinal plant used for gastrointestinal disorders. The ethanolic extract obtained from barks showed gastroprotective activity in healing gastric models. Bioassays with *Artemia salina* haven been shown to be a suitable pre clinical test of toxicity. The evaluation of the toxicity *in vivo* models that show biochemical profile and Histopathological analyses are essential. **Objective:** Evaluate the toxicity of the ethanolic barks extract of *Himatanthus sucuuba* in a *Artemia salina* bioassay, blood parameters and hepatohistological analyses. **Materials and Methods:** Barks from *Himatanthus sucuuba* were processed and *Artemia salina* bioassay were realized in the Laboratory of Pharmacology of Natural Products. Wistar rats (n=10) received the extract for 10 days (400 mg/Kg). Were observed the food and water ingestion, wight and exploration behavior. The animals were euthanized blood and hepatic samples were collected. Were evaluated glucose, cholesterol, triglycerides and creatinine from the blood and histological analyzes from the liver tissue. In the blood samples were dosage of gamaGT, AST e ALT, bilirubin and total protein. Liver samples were collected for histological analysis. **Results and discussion**: *Artemia salina* exposed to the extract at concentration of 1, 10, 100 and 100 showed survival rates similar to control. Behavioral analysis showed no difference from control. The biochemical evaluation showed no significant difference from control, nor the histological evaluation had difference from control. **Conclusion:** Results presented show a promising features by not showing toxicological alterations in the analyzed aspects.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 92- TOXICOLOGICAL ASPECTS OF THE DECOCTO FROM THE *HIMATANTHUS ARTICULATUS BARK*

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Introduction: The Amazon flora disposes of great diversity and many potentials medicinal plants. The decocto from the bark of *Himatanthus articulatus* are used in the state of Pará as anti inflammatory, anti gastric ulcer, and as anti cancer. Thus the study of toxicological aspects of this compound is of fundamental importance. **Objective:** Evaluate the toxicity of bioassay in the *Artemia salina* and electrocorticographic features of chronic administration of the lyophilized tea from the *Himatanthus articulatus* bark. **Materials and Methods:** *Himatanthus articulatus* samples were obtained from the Western Embrapa Amazon. Infusion were prepared from 100 g of dried bark, and later lyophilized. Bioassays were executed with larvae eclosed in the Laboratory of Pharmacology and Toxicology of Natural Products, realized in tiplice at concentration of 1, 10, 100 and 1000 ppm.. Electroencephalographic analyses: Wistar rats (n=10) received electrode implantation in the epidural space (Bregma -0.96 and 2 mm L). The animals were connected to a data-acquisition system (Amplifier, Grass Technologies P511; Digital acquisition, National Instruments P6112). All analyzes were carried out in a custom-made tool using Python language programming. The extract was administered for five days (3 mg/Kg) and then the electrocorticographic activity recorded (24, 48, 72, 96, 120 h). **Results and Discussion**: After 24 and 48 hours the larvae presented a survival of 80 %, similar to the control. The electrocorticographic records revealed no difference from control. **Conclusion:** The toxicological aspect showed a secure profile in the toxicologic profile analyzed.

EX 93- ELECTROCORTICOGRAPHIC CHARACTERIZATION OF PILOCARPINE-INDUCED SEIZURES IN NEONATES

LOBATO A.M.V.[1], CORDEIRO. P.G.A.[1], LOPES M.S.P.[1], NASCIMENTO L.N.S.[1], COSTA E.B.R.[1], BATISTA L. S.[1], MORAES R. S.[1],JÓIA-MELLO V.J.[1], HAMOY M.[1]

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Introduction: Pilocarpine is a natural alkaloid, presenting cholinergic agonism in muscarinic receptors. Clinical use is mainly via topical application. Systemic administration in high dose leads to seizures. The convulsive activity presentes many process which includes neurotoxicity, due to the excitotoxicity. This can have deleterious effects mainly in children and can be a step to epilepsy development. Nonetheless, seizure are more common in neonates and children than in adults. Clinical research suggests that seizures can have consequences in the long term and may even affect cognitive process in children.**Objective**: Analyze electrocorticographic profile of pilocarpine-induced seizures in neonates. **Materials and methods:** Wistar neonates rats (n=10) from Central bioterium of University Federal of Pará were submitted stereotaxic surgery, were implanted circular-tip on the motor cortex. The animals were connected to a data-acquisition system (Amplifier, Grass Technologies P511; Digital acquisition, National Instruments P6112) sampling rate of 1kHz and the data was hard-drive storage for offline analyzes. All analyzes were carried out in a custom-made tool using Python language programming. We recorded a basal period of 10 min followed by i.p. administration of 300 mg/Kg pilocarpine a 20% (Allergan ®) and recorded for 60 min. For controls we used aged-matched animals that receive the vehicle. (BIO243-14). **Results and Discussion:** Pilocarpine (300 mg/kg) induced seizures in electrocorticographic profile with increased amplitude of oscillations and cyclic potentials bursts. **Conclusion:** Pilocarpine induced changes in electrocorticographic profile according to convulsive activity in neonates.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 94- NEPHROTOXICITY EVALUATION OF ETHANOLIC BARK EXTRACT OF *HIMATANTHUS SUCUUBA*

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Introduction: The rising price and lack of full activity of allopathic medicines prompts the need to study and incorporate phytotherapy into the clinical practice. In order to obtain that a regulation by national regulator are needed. *Himatanthus Sucuuba* (HS), presents with various uses by the natives in the Amazon, most of the use relates to treatment of gastrointestinal conditions. In this light, the study of toxicological impacts of its administration are of essential importance. **Objective:** Evaluate nephrotoxic aspects of the administration of the HS ethanolic extract. **Materials and methods**: Barks from HS were collected and dried for the extract preparation. Wistar rats (n=10) received daily administration of the ethanolic extract (400 mg/Kg oral), a control group (n=10) received only vehicle for 10 days. Were observed food consumption, water ingestion, weight and exploratory activity. The animals were euthanized blood and renal samples were collected. Were evaluated glucose, cholesterol, triglycerides and creatinine from the blood and histological analyzes from the renal tissue. **Results and discussion**: Blood test, behavior analysis, and histological samples revealed no difference from the control group, showing no toxicity for the HS administration. **Conclusion:** Results presented show a promising features by not showing toxicological alterations in the analyzed aspects.

EX 95- ELECTROCORTICOGRAPHIC CHARACTERIZATION OF SEIZURES INDUCED BY CUNANIOL

MORAES R.S[1]; LOPES M. S. P[1]; NASCIMENTO L.N.S.[1]; CORDEIRO P.G.A.[1]; COSTA E.B.R.[1]; LOBATO A.M.V.[1]; BATISTA L.S.[1]; FARIAS R. A. F.[1], HAMOY M.[1], JÓIA-MELLO. V.[1]

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Introduction: Plants from the *Clibadium* genus are know the amazon region by its ictiotoxic features and are use in the fishing activity by riverine. Studies have related the convulsant properties in rats of the ethanolic extract and revealed the convulsant compound. Cunaniol is polyacetylenic alcohol isolated from plants of the *Clibadium* genus, a natural plant in the Amazon biodiversity, with promising properties as a convulsant model. Thus, we sought to describe the gamma band alteration during and 24 hours after the seizures induced by cunaniol in the attempt to further understand the underlying mechanisms and alterations induced by the cunaniol models. **Materials and Methods:** The botanical material was processed in the Laboratory of Pharmacology and Toxicology of Natural Products to produce the ethanolic extract of *Clibadium*. We implanted round-tip electrodes (Radius of 0.5mm) on the dura-mater over the motor cortex of adult Wistar rats (Bregma -0.96 mm AP and 2mm LL). In the experimental group the seizure was induced by administration of 150 mg of ethanolic extract of *Clibadium* v.o. We recorded the induced-seizure for 30 min. After 24 hours the animals were re-connected to the data-acquision system for recording of brain activity for 30 min. All the collected data was hard-drive storage for offline analyzes. The analyzes was carried out through a custom made toll using the Python programming language. **Results and Discussion:** Analyzes of the Gamma oscillation revealed a high increase in the oscillation range in the acute seizure, and relative reduction was observed in 24 hours. However, significantly higher than the baseline. **Conclusion:** The convulsant models induced by cunaniol can induce long-lasting alteration in the gamma band activity; which indicated a long-term effect. This observation talks in favor of a slow cunaniol metabolism or an alteration at the receptor level, due to the “acuteness” of the observed effects.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 96- COMPARASION BETWEEN ELECTRO-CORTICOGRAPHIC CHARACTERIZATION OF SEIZURES INDUCED BY CUNANIOL AND PENTYLENOTETRAZOLE

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Laboratory of Pharmacology and Toxicology of Natural Products – Institute of Biological Sciences – Federal University of Pará

Introduction: Cunaniol is a polyacetylenic alcohol isolated from the *Clibadium* genus, a plant found in the Amazon biodiversity, with promising properties as a convulsant. While the pentylenotetrazole is used with convulsivant model. As it is a model in development, we sought to describe the similarities and differences with a well-known convulsant model, the pentylenotetrazol. **Materials and Methods:** We collected the *Clibadium spp*. plants in the rural region of the Pará state. We obtained the ethanolic extract in the laboratory of Pharmacology and Toxicology of Natural Products. Wistar rats (n=10) under stereotaxic surgery we implanted circular tip electrodes in the motor-cortex region (Bregma – 1 mm AP and 2 mm LL). At the 7th post-op day the animals were connected to a data -acquisition system (Amplifier, Grass Technologies P511; Digital acquisition, National Instruments P6112). After a baseline, recording the animals received either ethanolic extract or pentilenotetrazol. **Results and Discussion:** Both models showed a similar activity with development of cyclic potential bursts and increment in the low-frequency range (1-50Hz) of the electrocorticogram. **Conclusion:** The seizures induced by the ethanolic extract of *Clibadium spp.* has high similarities to the seizures induced by pentilenotetrazol, which talks in favor to a similar seizure-underlying mechanism.

EX 97- CAFFEINE-INDUCED ELECTROPHYSIOLOGICAL CHANGES IN BRAIN OSCILLATIONS

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Introduction: Caffeine is the most widely consumed behaviorally active substance in the world. Almost all caffeine comes from dietary sources (beverages and food), most of it from coffee and tea. Caffeine is a phosphodiesterase inhibitor, which affects AMPc metabolism. The increased metabolism causes a neuro-stimulant effect, marked by sleepiness reduction, increase of the basal metabolism rate, motor activity, and in the blood pressure. The fast caffeine hepatic metabolism coffers low chronicity effect to the continued use of caffeine. Given the high consumption of caffeine in society, we sought to investigate the overall effects of caffeine in the brain oscillations. **Materials and Methods:** Wistar Rats (n=10) from Central bioterism of University Federal of Pará were submitted stereotaxic surgery. Under surgical procedures were implanted circular-tip electrodes (radius of 0.5mm) in the epidural space, coordinates of bregma -1mm and 2mm later-lateral. At the 7th post-surgical day the animals were connected to data-acquisition system (Amplifier, Grass Technologies P511; Digital acquisition, National Instruments P6112). Were recorded a baseline period of 10 min, followed by caffeine administration (10mg/Kg; Sigma-Aldrich). The data was sampled at 1 KHz and hard-drive storage for offline analyses. The data analyzes was carried out with a custom-made tool with Python programming language, with abilities to produce histograms, PSDs and spectrograms. **Results and Discussion:** Ours analyses reveals a significant increase of the low frequency oscillations in the brain (theta band, 4-10Hz) and decrease of higher frequency oscillations (gamma band, 20-50Hz). **Conclusion:** The increased excitability in the brain was demonstrated by the higher theta band activity and reduction of the gamma oscillations. These study describe the electroencephalographic profile (theta and gamma oscillations) associated of high-dose caffeine.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 98- ELECTROCORTICOGRAPHIC CHARACTERIZATION OF SEIZURES INDUCED BY ETHANOLIC EXTRACT FROM JAMBU (*ACMELLA OLERACEA* R.K. JANSEN)

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Introduction: Although the plants from the genus *Spilanthes spp.* has been long studied, little is known about the neurologic effects of its administration. Previous studies observed that administration of hexanic extract from *Spilanthes acmella* can induce tonic-clonic seizures in rats. Objectives: Characterize the seizures induced by the ethanolic extract of *Acmella oleracea* (L.) R.K. JANSEN. **Materials and Methods:** The ethanolic extracts were obtained in the Laboratory of Pharmacology and Toxicology of Natural Products through processing of roots collected in Belem-PA. Wistar rats (n=10) received electrodes in the epidural space (Bregma -0.96mm and 2mm L). In the seventh postsurgical day the animals were connected to digital acquisition system (Amplifier, Grass Technologies P511; Digital acquisition, National Instruments P6112) sampling rate of 1kHz and the data was hard-drive storage for offline analyzes. All analyzes were carried out in a custom-made tool using Python language programming. We recorded a basal period of 10 min followed by i.p. administration of 500 mg/Kg i.p the ethanolic extract and a control group received saline solution. **Results and discussion**: Electrocorticographic features shows a activity similar to a seizure activity and oscillations mainly in 2-5Hz during the seizure. A bigger power distribution is observed during the ictal activity. Latence for the first noticeable alteration was 423 ± 54 seconds. **Conclusion**: The ethanolic extract from *Acmella oleracea* induced electrocorticographic alterations compatible with toxicological profile seizure. The results suggest the ability of Jambu root extract to promoting the disruption of cerebral homeostasis.

EX 99- ACUTE AND SUBCHRONIC TOXICOLOGICAL EVALATION OF THE NOVEL PYRAZOLE DERIVATIVE LQFM021 IN MICE, RATS AND ZEBRAFISH

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INTRODUCTION: The new pyrazole LQFM021 was originally designed through molecular hybridization from milrinone and cilostazol. Previous studies has highlighted as a novel promising drug due to its possible phosphodiesterase -3 inhibitor effect associated to a strong vasorelaxant activity. In addition, it has showed antinociception effects mediated by peripheral opioid receptors with involvement of NO/cGMP/KATP pathway. Despite these interesting findings, toxicological profile of LQFM021 is not well known. **OBJECTIVE:** To evaluate the safety of the LQFM021 through acute and subchronic toxicological assays based on the validated guidelines from Organization for Economic Co-operation and Development (OECD). **MATERIALS AND METHODS:** After 28 days of treatment with LQFM021 (62.5, 125 or 250 mg/kg/day), female rats were euthanized for biochemical, hematological, histopathology analysis according to OECD 408. Micronuclei frequency in mice after exposed to the compound (300 mg/kg, 600 mg/kg, 900 mg/kg) was also investigated using flow cytometry (OECD 474). The cardiac toxicity in Zebrafish was performed according OECD 210. **RESULTS AND DISCUSSION:** LQFM021 did not change metabolic, hematological and biochemical parameters and no mutagenic potential were detected. However, the histopathological study indicated potential hepatotoxic and nephrotoxic of this compound, which deserves attention especially in larger doses during longer periods and even associated with other drugs. Preliminary results using Zebrafish showed that this compound didn't induce cardiac toxicity. **CONCLUSION:** These data add toxicological information for the novel pyrazole derivative LQFM021, which ensure future prospects in its use as a new antinociceptive agent mediated by peripheral opioid receptors.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 100- *Bidens pilosa* L. (ASTERACEAE) ASSOCIATED TO *Curcuma longa* L. (ZINGIBERACEAE) REDUCED INTESTINAL DAMAGE INDUCED BY CHEMOTHERAPY: MICRONUCLEI, HEMATOLOGIC AND ANTI-INFLAMMATORY EVALUATIONS

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Introduction: Intestinal mucositis is a serious dose-limiting side effect of chemotherapy that can promote interruption of the cancer treatment and hence reduce cure rates, increase therapy costs and decrease quality of life of the patient. Due to anti-inflamatory and anti-oxidant potential of *Bidens pilosa* (BP) and *Curcuma Longa* (CL), respectively, both plants have showed interesting results to treat 5-fluorouracil (5-FU)-induced mucositis in mice. In view of these, we developed a mucoadhesive formulation based on poloxamer 407 containing BP and CL (FBC) to improve the protective effects of these natural products. **Objective:** To evaluate the therapeutic role of FBC in the hematologic and inflammatory parameters of mucositis -bearing mice. Moreover the frenquency of micronuclei was also investigated after exposure to this formulation. **Material and Methods:** FBC was composed by CL (1%, m/m) and BP (40%, v/v) extracts, poloxamer 407® (15%, m/m), Surplus® HS15 (3.2%, m/m), Transcutol HP (10%, v/v), citric acid (to pH 4.5-6.0) and polyethylene glycol 400 as a liquid vehicle. FBC at 125 mg/kg BP + 15 mg/kg CL dose was administered in Swiss male mice with or without 5-FU-induced intestinal mucositis during 6 days. At 7th day, blood collection and small intestine removal of each animal were carried out for red and white blood cells counting and measurement of anti-inflammatory IL-10 cytokine, respectively. Micronuclei was performed according to OECD 474. **Results and Discussion:** 5-FU group showed a reduction of 18.0 and 31.2% in red and white blood cells, respectively, in comparison to control. Contrasting, the treatment of mice with FBC was able to promote an increase of 10.0 and 30.5% in these same parameters when compared to 5-FU group, demonstrating the hematoprotective potential of this mucoadhesive formulation. In the inflammatory response evaluation, it was observed that animals exposed to 5-FU only showed a decrease of 80.2% for IL-10 levels, when compared to control. In contrast, values found for mucositis-bearing mice, treated with FBC, were close to those found in the control group. Exposure to BBC didn't change the micronuclei frequency. **Conclusion:** Pharmaceutical technology used in FBC improved the protective properties of BP and CL in the treatment of 5-FU-induced intestinal mucositis, due to anti-inflammatory and hematoprotective potential effects with no changes in the micronuclei frequency. Given the perspectives for development of a new medicine, further studies are in progress to better understand the therapeutic effects of this innovative formulation.

Acknowledgements: FAPEG, FUNAPE-UFG, CNPq, FINEP and CAPES.

EX 101- GENE EXPRESSION ASSESSMENT IN THE LIVER OF WISTAR RATS TREATED WITH THE EXOPOLYSACCHARIDE LASIODIPLODAN

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Introduction: The lasiodiplodan is a β-(1→6)-D-glucan produced by the fungus *Lasiodiplodia theobromae* (MMPI). Some of the biological activities of this exopolysaccharide were reported as hypoglycemic, anticoagulant, antiproliferative and anticancer. Previous studies carried out in Wistar rats have shown that this exopolysaccharide (i) does not induce genotoxicity (comet assay) and mutagenicity (micronucleus test); (ii) in the treatments associated with the antitumor doxorubicin (DXR), lasiodiplodan showed a protective effect against DXR-induced DNA damage. **Objective:** The aim of this study was to evaluate the expression of 84 genes associated with the DNA damage pathway in order to further explore the mechanisms of protection previously observed in the liver of Wistar rats. **Materials and Methods:** The treatment was performed by the administration of water (control group) or 10 mg/kg of lasiodiplodan by gavage for 14 days; followed by the injection of saline or the antitumoral DXR (15 mg/kg) just after the last gavage, intraperitoneally. The animals (n=3/group) were euthanized 24h after the last gavage. Gene expression was evaluated by RT^2 Profiler^{TM} PCR Array in the liver. **Results:** The lasiodiplodan alone significantly increased the expression of *Tp53* and *Bax* genes. DXR alone induced an increase in the expression of 5 genes and decreased the expression of 9 genes, evidencing its effects on DNA repair and DNA-damage signaling. More important, our results demonstrated that animals treated with DXR associated with lasiodiplodan did not show changes in gene expression profile, showing gene expression pattern similar to those from control group. **Conclusion:** In conclusion, associated with our previous results with DNA damage, the data presented herein suggest that lasiodiplodan exerts strong protective effect against DXR-induced DNA damage. However, further *in vitro* and *in vivo* studies are still necessary to elucidate these mechanisms.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EX 102- STUDIES ON THE CHARACTERS AND MECHANISMS OF TESTICULAR TOXICITY INDUCED BY HYDROXYUREA

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Objective: Apoptosis plays a dominant role in both spontaneous spermatogenesis and germ cell death. This study was aimed to investigate the functions of related genes in testicular germ cell death induced by Hydroxyurea (HU). **Method:** Wild-type (WT) and FasL transgenic (TG) DBA/C57BL mice were intraperitoneal injected with 400 mg/kg HU. 12 h later, testes were collected. Histomorphology of testis was observed by stained with Periodic Acid Schiff (PAS).Apoptosis was assessed by TUNEL assay. mRNA and protein levels of related genes was evaluated by quantitative RT-PCR and Western blot, respectively. **Results:** The 2×2 factorial design comparative experiments between WT and TG mice showed that the TG mice exhibited a higher basal apoptotic index. The basal mRNA levels of Fas and FasL and protein levels of Fas, FasL, Caspase-3, Caspase-8 and Caspase-9 in TG mice were also higher than that in WT mice. 12 h after injection of HU, the testicular tubules exhibited no significantly morphological changes but remarkably increased apoptosis index in both WT and TG mice, with the latter having the higher amplitude. Although HU up-regulated the mRNA of apoptosis related genes such as Fas and FasL in both TG and WT mice, the increased amplitude were more obvious in TG mice. By Western blot analysis, apoptosis related proteins Fas, FasL Caspase-3, Caspase- 8 and Caspase-9 were significantly increased in both WT and TG mice, with TG mice exhibiting a greater up-regulation. **Conclusion:** Germ cell apoptosis induced by HU treatment may be related to the FasL mediated signal transduction pathway.

This work was supported by Shanghai experimental animal scientific and technological innovative action plan （No:14140901302）; Shanghai professional service platform of non-clinical evaluation of drug against male reproductive and urinary diseases（No: 15DZ2290400）

EX 103- ESTABLISHMENT OF PROSTATE CANCER IN CYNOMOLGUS MACAQUE ANIMAL MODEL BY ORTHOTROPIC INOCULATION OF PC-3 CANCER CELLS IN SITU

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Background Prostate cancer commonly affects men worldwide. Till now, no suitable animal models which could fully embody the characteristics of human prostate cancer due to different deficiencies are still available. This investigation explored the potential application of cynomolgus macaques to establish prostate cancer animal model which is more similar to the characteristics of human disease. **Methods:** Four 4-year-old cynomolgus macaques were used in this study. Cyclosporine conducted as immunosuppressant was subcutaneously injected to cynomolgus macaques once daily. Following 7 days of treatment of cyclosporine, 1×10^7 PC-3 cancer cells mixed with matrigel were injected into the prostate of two cynomolgus macaque, while the other two cynomolgus macaque were injected by culture medium at the same site as the mock group under anaesthesia state. Cefazolin Sodium acted as antibiotics was administered through intramuscular injection for five days after the operation in protection of bacterium infection. **Results:** The serum concentration of PSA mildly increased in the two PC-3 cancer cells treated monkey on day 30 and 60 although neither the serum concentration of ALP nor body weight was significantly affected. Prostate cancer has developed in two monkeys following PC-3 cancer cells inoculation. It was shown typical prostate cancer could be observed in histopathological examination. These tissue sections revealed that prostate cancer cells had invaded the nerves, the basal layer was discontinued and the developed tumor lesions had reached the grade II Gleason lesion level. Furthermore, the detection of anti-human α-methylacyl coenzyme A racemase (p504s), p63 and basal keratin antibodies (34βE12) by immunohistochemistry also demonstrated that cancer had developed. However, there was no evidence of prostate carcinoma metastasis occurred in the two monkeys. The gene expression profiling analysis also indicated that most human prostate cancer genes were expressed in the prostate tissue of the monkeys. **Conclusions:** In this study, we have established a cynomolgus macaque model of human prostate carcinoma by an orthotropic injection of PC-3 cancer cell line in situ. These results demonstrated that this model may effectively simulate the biological nature of human prostate carcinomas.

This work was supported by Shanghai experimental animal scientific and technological innovative action plan （No:14140901302）; Shanghai professional service platform of non-clinical evaluation of drug against male reproductive and urinary diseases（No: 15DZ2290400）

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

IMMUNOTOXICOLOGY

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

IM 01- HER-1 CANCER VACCINE: IMMUNOTOXICOLOGICAL STUDIES FROM NON-CLINICAL EVALUATION IN NON-HUMAN PRIMATES TO CLINICAL EVALUATION IN PROSTATE CASTRATION-RESISTANT CARCINOMA PATIENTS.

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Introduction: Epidermal growth factor receptor (HER-1) constitutes a tumor associated antigen. It is overexpressed in many epithelial tumors, including prostate cancer, and has been associated with bad prognosis and poor survival. Cancer vaccine based on the extracellular domain (ECD) of HER-1 adjuvated in very small sized proteoliposomes (VSSP) and Montanide ISA51-VG is a new and complementary approach for the treatment of epithelial tumors. The present studies deal with the toxicity and immunogenicity of this vaccine in Macaca fascicularis monkeys and prostate-castration-resistant carcinoma patients. **Materials and methods:** Twelve monkeys were randomized into two groups, control and vaccinated (200 mg). Treated monkeys received 9 doses of vaccination in 6 months and were inspected for clinical signs during a year. Vital signs were measured during the study. Humoral immune response, clinical pathology parameters were analyzed. Skin biopsy was performed at the end of the study in all animals. Patients were enrolled in a dose-escalation clinical trial design, in which 5 doses of vaccine, 5 patients per group (from 100 to 800 mg) were tested. **Results and discussion:** Animal's survival in the study was 100%. Local reactions were observed at the administration site of four treated animals, two showing slight inflammatory cutaneous damage. Clinical pathology parameters were not affected. HER-1 vaccine induced high IgG antibodies titers in treated animals. Cancer patients also received 9 doses of vaccine in 6 months and were observed for clinical pathological signs, hematological, immunological and biochemical parameters during a year. The most common adverse events were pain at injection site, asthenia, flu-like symptoms, and fever. There was an increase in the overall survival in relation with the dose level. **Conclusions:** HER1 vaccine is safe and well tolerated; it induces high titers of anti-HER1 antibodies in monkeys and humans and a phase I/II clinical trial in different localizations is on the roadmap.

IM 02- THE EFFECT OF CRACK COCAINE SMOKE EXPOSURE ON RATS CHALLENGED WITH KEYHOLE LIMPED HEMOCYANIN

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The crack cocaine abuse is a public health concern worldwide and despite many inconsistent studies related to the immunotoxic effect of cocaine hydrochloride in in vitro and in vivo models, due to differences among lab species, concentrations and the route of administration, there are many epidemiological studies revealing a higher prevalence of infectious diseases in crack users. However, no immunotoxic study with this drug in animal model was yet performed. Thus, the present study aimed to evaluate the acquired immune responses of rats exposed to the smoke of crack cocaine, twice a day, during 28 days. For that, 20 Wistar rats were divided into 2 groups, one exposed to the smoke of 250mg of crack cocaine burned into a pipe during 10 minutes (blood cocaine level≈174ng/mL/exposition), twice a day, for 28 days (CK group) and one sham control group, which was exposed to burned air. In addition, due to the cocaine anorectic effects, it was also performed a pair-fed (PF) group (n=10) which received the same amount of food consumed by animals from the CK group to rule out any possible malnutrition effect on the immune responses. On experimental day (ED) 14 and 21, all rats were sensitized with 0,1mL of Keyhole Limped Hemocyanin (KLH) (1mg/mL; sc). On the ED 28, they were challenged with 0,1mL of heat-aggregated KLH on the foot paw (10mg/mL; id). After 24h, the tumor paw was measured (Delayed-Type Hypersensitivity – DTH assay) and blood was collected for anti-KLH IgM and IgG evaluation (T-dependent antigen response – TDAR assay). In relation to food consumption, as predictable, it was observed a reduction on the food intake of animals exposed to the drug, which effect was transient along the experimental period of evaluation, resulting in a reduction of 8% of total food consumption when compared to the control group. In relation to the TDAR, no statistical differences among the groups were observed; however, when the DTH was evaluated, it was observed a statistical reduction on the tumor paw size of animals from the PF group when compared to the control and the CK group. This effect could not be resulted from the 8% of food restriction, since similar effect was not observed on CK group. What could be causing this effect? Importantly, both CK group and control group were exposed to carbon monoxide (CO) while the PF group did not. Is it possible to conjecture a possible immunomodulatory effect of CO on the immune function of these animals? In fact, studies have shown that CO has several effects on immune cells. Despite the failure to observe any immunotoxic effect of crack on immune responses here evaluated, there is no doubt that other studies will be conducted to better understand the CO effect on the immune system.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

IM 03- IMMUNOMODULATORY EFFECTS OF SUBTOXIC METAL SALT CONCENTRATIONS

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Background: Exposition towards metal salts plays a major role in Environmental Medicine. Toxic effects and toxicological limits are quite well known but effects at subtoxic concentrations have not yet been investigated in detail. **Methods:** Toxicity of 8 metal salts ($Pb(NO_3)_2$, $AuCl_3$, $Cu(NO_3)_2$, $HgCl_2$, $AgNO_3$, $SnCl_2$, $AsCl_3$, $SbCl_3$) towards human peripheral blood nononuclear cells (PBMC) was analyzed using proliferation-, (WST), apoptosis (Annexin) and necrosis (Propidiumjodine) assays and the respective sodium salt anion as control. Genotoxicity (Comet assay), proliferation (CFSE assay) and cytokine production (Interleukin-2, Interferon-γ) of lymphocytes as well as maturation of dendritic cells (DC) were investigated *in vitro* at subtoxic concentrations. **Results:** Non-toxic concentrations of $Pb(NO_3)_2$, $AuCl_3$, $Cu(NO_3)_2$, and $AsCl_3$ had distinct antiproliferative effects on human lymphocytes; $Pb(NO_3)_2$, $AuCl_3$, $Cu(NO_3)_2$, $HgCl_2$ and $AsCl_3$ inhibited IL-2-production, and $Pb(NO_3)_2$, $AuCl_3$, $Cu(NO_3)_2$, HgCl2, $SnCl_2$, $AsCl_3$ and $SbCl_3$ inhibited the release of Interferon-γ. No effects on maturation of DC and no genotoxicity could be found. Effects of $Cu(NO_3)_2$, $HgCl_2$ and $AsCl_3$ on PBMC were found at concentrations which are regarded as normal or safe human serum concentrations. **Conclusion:** Subtoxic concentrations of metal ions have different inhibitory effects on human lymphocytes *in vitro* which should be further analyzed in animal studies and in humans.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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A | R | T
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FO 01- CHEMICAL PROFILE OF VOLATILE ORGANIC COMPOUNDS (VOC'S) USED AS INHALANTS SEIZED AT THE BRAZILIAN CARNIVAL

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Introduction: The terms "inhalants" and Volatile Organic Compounds (VOC's) are used to describe a wide range of volatile chemicals that may be inhaled accidentally or intentionally. The extensive availability and low cost of inhalants have contributed to an increased incidence of intentional inhalation of volatile substances during popular festivals in Brazil. During the Carnival of Bahia, in 2015, more than 60 glass ampoules were seized by local police. According to the National Agency of Sanitary Surveillance (ANVISA), the marketing and the recreational use of these substances is illegal under Resolution 344/98 which lists the prohibited substances. However, some narcotic substances that are not prescribed are being illegally used in an attempt to circumvent the law. **Aims:** The objective of this work is to present the chemical profile of inhalants seized during the Carnival of Bahia in 2015 and the occurrence of new volatile substances used as drugs of abuse that are not proscribed in accordance with brazilian law. **Methods and materials:** Aliquots of 500 uL of fluid were collected from each glass vial through the original spray bottles and transferred to the headspace vials (20 mL). The samples were injected (2.5 mL) and analyzed by HS-GC-MS in order to carry out screening and identifying volatiles compounds such as ethanol, ethyl chloride, methylene chloride, diethyl ether and others fluorinated and chlorinated organic solvents commonly have arisen in such samples. **Results:** 61 samples were analyzed and 13 different volatile organic compounds were identified, with prevalence of halogenated substances and more than one volatile compound per sample. Prevalence of identified compounds were as follows: Ethanol 45,9% (n=28); Hexane 1,6% (n=1); Diethyl ether 3,3% (n=2); Dimethyl ether 16,4% (n=10); Ethyl chloride 6,5% (n=4); Methylene chloride 75,4% (n=46); Chloroform 30% (n=18); Dichlorofluoroethane 41% (n=25); Difluorochloromethane 5% (n=3); Chloromethane 3,3% (n=2); Dichloroethene 1,6% (n=1). **Conclusions:** It was observed the prevalence of halogenated substances containing fluorine and chlorine, especially methylene chloride and dichlorofluoromethane used as refrigerant (called HCFC 141b). The latter substance appears in 41% of the samples and it is not illegal under Brazilian law. However, this substances cause cardiac arrhythmia in potential and depression of the central nervous system (CNS).

FO 02- IDENTIFICATION OF NOVEL PSYCHOACTIVE SUBSTANCES (NPS) IN SEIZED MATERIALS USING GC-MS, FTIR AND NMR: PREVALENCE OF CATHINONES AND PHENETHYLAMINES

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Introduction: The term “Novel Psychoactive Substances” (NPS) is used to define narcotic substances or psychotropic drugs that are not scheduled under the United Nations 1961 or 1971 Conventions, but which may pose a threat to public health comparable to scheduled substances. In Brazil the two major classes of these compounds that have emerged are the cathinones and phenethylamines in different forms such as tablets, crystals and blotter paper. Considering the difficulties encountered in the acquisition of reference materials of such substances, an important alternative is to isolate and characterize these compounds using spectrometric techniques. We isolated three compounds in relatively pure samples and characterized them using GC-MS, FTIR and NMR data. **Aims:** The objective of this work is to present the complete structural identification of NPS that was identified in seized materials with the prevalence of cathinones and phenethylamines. **Methods and materials:** Were analyzed two crystals samples and a small tablet which were seized by the Bahia State Police. The active ingredients of samples were extracted with 500 uL of methanol, taken to ultrasound for 15 min and 1 µL of the extract was injected into the GC-MS. A fraction of a tablet (about 500 mg) were pulverized, added to the microtube with 1 mL of methanol, brought to ultrasound for 15 min and centrifuged. The supernatant was removed and transferred to a 2 ml vial, brought to dry under a stream of N_2 and weighed on an analytical balance. This step was repeated until obtaining a mass of about 15 mg. It was solubilized in D_2O and taken for analysis by NMR. A small fraction of the dry extract was analyzed by ATR-FTIR. **Results and dicussion:** Due to the unavailability of certified reference material in Brazil for the vast majority of NPS, identification was based on the fragmentation profile obtained by EI-GC-MS together with NMR and FTIR spectra. Preliminary screening using GC-MS with updated libraries indicated the presence of methylone and ethylone in crystals and clobenzorex in tablets. The confirmation of the structure of these compounds was achieved by analysis of the FTIR spectra and assignment of functional groups, characteristic absorption bands in the wavenumber range of 4000-600 cm^{-1} and chemical shift assignments in ^{1}H NMR and ^{13}C NMR Spectra using a NMR Bruker 600 MHz Spectrometer. **Conclusions:** The methylone, ethylone and clobenzorex have been completely identified. The materials analyzed with spectra acquired were admitted as secondary reference material to confirm the identity of other seizures. These materials will be used in the framework of the scientific police to conduct forensic analysis.

References:

[1] Strano-Rossi, S. *et al*; *Rapid Commun. Mass Spectrom. 2014, **28**, 1904–1916*

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

FO 03- DETECTION OF ETHANOL IN BRAZILIAN GASOLINE STATION ATTENDANTS

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Introduction: Millions of people die from traffic accidents, and a large proportion of accidents related to driving under the influence (DUI) of alcohol or drugs. In Brazil, gasoline station attendants are exposed daily during the workday, to ethanol in the fuel and ethanol used as an additive in gasoline. **Objective:** This study aimed to assess the potential exposure of gasoline station attendants to this component, using breathalyzer and oral fluid (OF) analysis by headspace gas chromatography/mass spectrometry (HS-GC/MS). **Materials and methods:** Gasoline stations attendants of 26 gasoline stations were invited to participate in the study. 162 gasoline station attendants were invited to respond a questionnaire covering the main features of the study population and the profile of drinking and driving behavior, followed by a breath test and OF collection, which were analyzed by HS-GC/MS. **Results and discussion:** Ethanol was found in 100% of the samples, and 72.83% of samples had concentrations above the quantification limit of the method (0.00125 g/dL). Regarding the breath tests, only one sample (0.62%) had a positive result (0.03 mg/L). Despite the low concentrations found in OF of gasoline station attendants, ethanol concentration, in this matrix, depends on ethanol concentration in the blood. However, in some traffic legislations the limit allowed for ethanol in the blood of drivers is equal to zero (e.g., in Brazil) so that any level of ethanol detected may ultimately generate legal complications for the driver. **Conclusions:** The results presented here demonstrate that the breathalyzer is less effective when compared to OF analysis by HS-GC/MS, showing the importance of confirmatory analysis for the breathalyzer.

Acknowledgments: CNPq for financial support and scholarships, State Traffic Department of Rio Grande do Sul (DETRAN-RS) by lending the breathalyzer, and Immunalysis Corporation for donation of Quantisal™ OF collection devices.

FO 04- CANNABINOIDS IDENTIFICATION BY ESI(–)-FT-ICR MS AND ESI(+)-FT-ICR MS

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Introduction: *Cannabis sativa* L., popularly known as marijuana, is one of the oldest plants that man is aware, and is historically the most commonly used illicit drug worldwide. An annual and dioecious plant, is chemically characterized by presenting more than 500 constituents, in which cannabinoids are the class of more interest for being considered the most responsible for the biological activity of the plant. About 100 cannabinoids are known, of which the principal psychoactive substance that presents toxicological interest associated to the abuse of drugs is Δ9-tetrahidrocanabinol. Electrospray ionization (ESI), a soft ionization and no fragmentation technique, and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) provides the highest available mass, mass resolving power and mass accuracy, has been applied in a number of sciences including metabolomics, proteomics, and petroleomics. The high mass accuracy allows define unambiguous elemental composition by just their mass-to-change ratio (*m/z*) and singly charged. Also, double bond equivalent (DBE) has been defined by this high accuracy, facilitating molecule classification by heteroatom content and the degree of aromaticity. **Objective:** The aim of this study was the identification of cannabinoids through the ESI(–)-FT-ICR MS and ESI(+)-FT-ICR MS. **Materials and methods:** 15 mg of leafs of 50 cannabis plants were extracted with 1.5 mL of acetonitrile and were placed in an ultrasonic bath for 10 min. After the samples were diluted to ≈ 1mg mL^{-1} in acetonitrile which contained 0.1% m/V of NH_4OH for ESI(–) and 0.1% m/v of HCOOH for ESI(+). FT-ICR MS analyzes were performed using the mass spectrometer, was set to operate over a mass range of *m/z* 154-1250. **Results and discussion:** ESI(–)-FT-ICR MS spectra showed lower number of signals compared to ESI(+)-FT-ICR MS. Furthermore, while in the negative mode the signals were detected in the range of m/z 250 to 750, in the positive mode the signals were detected in the range of m/z 250-900. FT-ICR MS analyzes in both ionization mode was possible to observe the presence of molecules identified as monomers and dimers. Unlike the negative mode, the analyzes in positive mode did not show characteristic chemical profile for cannabis and that can be explained by the presence of adducts as sodium $[M + Na]^+$ and potassium $[M+K]^+$. **Conclusion:** Although the analyzes were performed in both ESI mode, only the negative mode showed a characteristic profile of cannabis as already reported in the literature. The presence of adducts was inappropriate factor in interpreting the results in positive mode.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

FO 05- LIQUID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF ANTIDEPRESSANTS IN VITREOUS HUMOR: STUDY OF MATRIX EFFECT OF HUMAN AND BOVINE VITREOUS AND SALINE SOLUTION

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Introduction: Vitreous humor (VH) is a very important biological matrix in the field of Forensic Toxicology. Its properties and uses have been known for a long time and in the postmortem cases is possible the identification of illegal and therapeutic drugs. However, few studies with antidepressants in VH are available. In addition, the matrix effect in the validation of methods using gas chromatography-mass spectrometry (GC-MS) is an issue that requires further information. **Aims:** Develop a new method using a hollow-fiber liquid-phase microexctration (HF-LPME) with the antidepressants (amitriptyline, nortriptyline, imipramine, desipramine) by GC-MS and ensuring the presence or absence of matrix effect. Furthermore, provide more data about the relationship between antidepressants in VH and femoral whole blood (FWB). **Methods:** An aliquot of 0.50 ml of VH sample (or saline solution – 0.9% NaCl) was transferred into a 5-ml glass tube containing a 10-mm magnetic stirrer, followed by 3.5 ml of 0.1 M NaOH solution. Deuterated ISs were added to this sample solution: 10 ng of AMI-d_3; 100 ng of DES-d_3, IMI-d_3, and 200 ng of NTR-d_3. Two hollow fibers with 8-cm each, whose pores were filled with dodecane (organic phase), was used for each extraction. The lumen of the hollow fiber was filled with 30 µl of 0.1 M formic acid (acceptor phase) using a micropipette, and the filled hollow fiber was placed into the sample solution in a U-shaped configuration. During extraction for 10 min, the solution was stirred at 1,000 rpm at 55ºC. After extraction, the acceptor phase was withdrawn from the fiber using a thin tip and dried under a nitrogen stream at 40ºC. The residue was suspended in 30 µl of methanol. No derivatization step was required. Subsequently, 2.0 µl of the solution was injected into the GC–MS system, with the ion source voltage altered to 50eV. **Results:** Matrix effect was evidenced imipramine and desipramine. The two other antidepressants, presented performances that allowed complete absence of this phenomenon and we were able to validate the method using saline. The limits of quantification and qualification were, respectively, 5.0 and 1.0 ng/ml of antidepressants in VH. The calibration curves were linear over the specified range (5 to 200 ng/ml; r^2>0.99). The recovery of the analytes was in average 78%. Precision values, inter- and intra-days, were better than 15% relative standard deviation (RSD). Accuracy was over than 85%. An average ratio (VH/FWB) of approximately 0.1 was found for both compounds. In the MS equipment, lower ionization voltage provided highest sensibility in the method. **Conclusions:** The matrix effect should be considered in forensic toxicology, even in methods based on GC-MS. Even analytes with similar chemical structures may exhibit different behaviors. The LPME method developed for the determination of AMI and NTR in VH proved to be appropriate for the analysis of real *postmortem* cases.

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FO 06- FAST DETECTION OF COCAINE IN DRUG SEIZURES BY CAPILLARY ELECTROPHORESIS/ TANDEM MASS SPECTROMETRY

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The abuse of addictive drugs had increased worldwide, causing serious social problems. In order to protect human health and to comply with strict legislation, the development and the application of analytical methods of drugs of abuse are very important. Cocaine is frequently seized mixed with a wide variety of adulterants such as benzocaine, lidocaine and caffeine, as well other substances carefully chosen to specifically hide the physical characteristics of the cocaine and to difficult its detection[1]. Moreover, due to forgery, drug addict can suffer overdose and even death. The forensic identification of cocaine in drug mixtures is usually performed by colorimetric testing kits, but these tests may suffer from interferences, producing wrong results. This work aims to use capillary electrophoresis coupled tandem mass spectrometry (CE-MS/MS) as a fast method to detect cocaine in drug seizures with adequate sensitivity and selectivity. The CE-MS/MS analysis was performed on an Agilent 7100 CE system coupled to Agilent 6430 Triple Quadrupole mass spectrometer. The cocaine separation by capillary electrophoresis was achieve using 30 mM ammonium formate, pH 4.5, as background electrolyte (BGE) and 5 mM methanol-ammonium formate (50:50 v/v methanol/water) , pH 6.5, as the sheath liquid at a flow rate of 6 µL/min. The CE system was operated at 26 kV, 25 ºC, using a 50 µm i.d. 80 cm long fused-silica capillary. The samples (10 mg) were dissolved in water (10 mL), filtered, and injected at 100 mBar by 12 s. The acquisitions were performed using positive polarity mode in two unambiguous transitions for cocaine (*m/z* 304 à 182 and *m/z* 304 à 82), drying gas temperature at 160 ºC (N_2), gas flow at 8 L/min, nebulizer pressure at 10 psi, and capillary voltage at 5000 V. The limit of detection was determined to be 0.3 µM and the analysis time was less than 5 min per sample. The method presents excellent precision data for replicate injections, and it was successfully applied to the detection of cocaine in drug seizures samples.

1. M.J. Binette & P. Pilon, Microgram J **10**, 8 (2012).

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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FO 07- DETECTION OF COCAINE, AMPHETAMINE AND TETRAHYDROCANNABINOL IN ORAL FLUID SAMPLES FROM TRUCK DRIVERS IN THE STATE OF SÃO PAULO, PRELIMINARY FINDINGS

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Introduction: In Brazil, traffic accidents were responsible for more than 43 thousand deaths in 2013, causing a significant economic and social impact. About 90% of all goods produced are transported on highways. It is already known that psychoactive substances may reduce the driver's capability to drive a vehicle safely. Truck drivers represent a risk group for the use of drugs, because many of them are driving long distances with little resting time, often during the night. Brazilians studies have already demonstrated a frequent use of amphetamine, cocaine and cannabis by truck drivers through toxicological analyses of urine. However the main international studies on the use of drugs by drivers have used oral fluid as biological matrix. The presence of drugs in oral fluid reflects the presence in blood at the time of sample collection. In addition, the collection is easy, not invasive, and it is almost impossible to adulterate. Considering these facts, we choose to introduce oral fluid samples in our studies to confirm recent use of drugs by truck drivers. **Aims:** The aim of this study was to screen oral fluid samples collected from truck drivers in the state of Sao Paulo with the ELISA method to detect the presence of amphetamine, cocaine and cannabis. **Methods:** The samples were collected between March 2014 and March 2015. The drivers included on this study were participating in health preventive actions carried out by the Federal Road Police, entitled "Health Commands on the Roads". Besides the collection of oral fluid, we also recorded socio-demographic and occupational data by using a structured questionnaire in order to study any association with the toxicological results. The oral fluid samples were submitted to ELISA testing for the detection of drugs. **Results and Discussion:** 764 drivers were stopped and 762 agreed to participate. All drivers were men with an average age of 42.5 years. The majority was married (71.1%), and the drivers had studied an average of 8 years in school. 67.7% of the participants had contract with a transportation company for 15.6 years of average. On the ELISA test, 52 samples were considered positive for at least one drug. The most frequently detected substance was cocaine (3.01%), followed by amphetamine (2.49%) and cannabis (1.31%). 5 samples were positive for more than one drug (2 cocaine + amphetamine, 2 cocaine + cannabis and 1 amphetamine + cannabis). Our results are in accordance with other Brazilian studies involving toxicological analysis on truck driver's oral fluid. All the positive samples will be confirmed by GC-MS after SPME extraction. **Conclusion:** The use of psychoactive substances by truck drivers is still frequent and cocaine is the most used drug among truck drivers.

FO 08- COHb CONCENTRATION AND OTHER DATA FROM CHARRED CHILDREN´S BODIES AUTOPSIED IN THE INSTITUTE OF LEGAL MEDICINE OF SAO PAULO, BRAZIL

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Introduction: The incomplete combustion of materials generates carbon monoxide (CO). After pulmonary absorption, CO forms a stable bond with the hemoglobin in the red blood cells, shifting the equilibrium and forming carboxyhemoglobin (COHb). This process impairs oxygen binding to the red blood cells, resulting in death by hypoxia. In forensic cases, the interpretation of COHb saturation levels could be defined as follows: values above 50% COHb have been usually consistent with death. When values are between 10% and 50% COHb, there is indication of inhalation of some smoke, which could have contributed to death, or at very least, that the individual was alive when the fire begun. Values below 10% COHb could be suggestive of the fact that the individual was dead before or died soon after the fire started. However, the elderly and children can have a low tolerance for CO poisoning; therefore, they can die despite relatively low blood concentrations of COHb. **Aim:** The aim of this study was to analyze the data from charred corpses of children autopsied at the Institute of Legal Medicine of Sao Paulo, Brazil, and correlate this data with the COHb concentration and soot in the airways. **Methods:** Data from the autopsy reports from eight charred corpses were analyzed. Only children younger than 10 years were included in this study. The COHb blood levels were was analyzed by spectrophotometric method with reduction by sodium dithionite. **Results and discussion:** In all cases of children included on this study, the cause of death was given as CO poisoning. Soot was found in the airways of all those victims. The COHb concentration ranged from 15.2% to 95.6%. However, only three cases presented COHb above 50%. Considering the reference values found in the literature, the cause of death should be given as CO poisoning only in cases presenting more than 50% of COHb due CO poisoning. **Conclusion:** With this study, we observed the importance to evaluate the concentration of COHb and the presence of soot in the airways in charred bodies. It is also important, to correlate these variables with the age of the victim. This correlation may indicate that death due CO poisoning can occur with lower levels of COHb than those describes in literature, especially on children.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

FO 09- VALIDATION OF AN ANALYTICAL PROCEDURE FOR DETERMINATION OF THC METABOLITE IN URINE SAMPLE FOR OCCUPATIONAL EVALUATION

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Introduction: The Δ9-tetrahydrocannabinol (THC) is the main cannabinoid with psychoactive effect. Among the main psychoactive effects of THC euphoria, feelings of relaxation and well-being stand out. In addition, psychomotor ability may be reduced enough to affect the performance on the direction of automotive vehicles and in work activities. As the main major metabolite of THC excreted in urine is the acid 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC COOH), the use of cannabis is confirmed by quantifying the urinary metabolite THC-COOH, with a cutoff value greater than 15 ng.ml^{-1} according to Substance Abuse and Mental Health Services Administration (SAMHSA). **Objective:** This study aimed to determine THC-COOH in urine by GC-MS using liquid-liquid extraction for occupational assessment. **Materials and Methods:** Was utilized a GC-MS PerkinElmer Clarus SQ 8T with Agilent column HP-5MS (30 m x 0.25 mm x 0.25 µm). The chromatography method developed has 10 min, starting at 160 °C and holding for 2 min, with a heating ramp up to 300 °C at 20 °C.min^{-1} and Helium flux of 2.0 mL.min^{-1}. The injection was done in splitless mode and 0.75 min with the injector at 260 °C. The mass spectrometer operated at 250 °C in the electron impact mode with energy of 70 eV. The quantification was performed in selective ion monitoring mode with the ions 371 (quantifier), 473, 488 m/z (qualifiers) for THC-COOH and deuterated internal standard (IS) THC-COOH-d3 are 374 (quantifier), 476, 491 m/z (qualifiers). The procedure of extraction comprises add 1.0 mL of urine sample in a 10.0 mL test tube, 50 µL solution of IS and 100 µL of KOH 10.0 M. Hold at 60 °C in an oven for 20 min. Adjust the pH to 4 – 6 with Glacial Acetic Acid. Add 2.0 mL of solution Hexane:Ethyl Acetate (90:10), stiring for 30 sec, collect 1.4 mL of the supernatant and drying under vacuum at 60 °C. For derivatization, was added 50 µL of BSTFA:Pyridine:TMCS solution (89:10:1) and hold in oven at 70 °C for 25 min. It injects 1.0 µL in the chromatographic system. **Results:** The method is linear in range from 6.0 to 192.0 ng.mL^{-1}. The method presented intra- and inter-day precision, ranging from 0.2 to 4.3%, and 2.0 to 6.9%, respectively, and accuracy of 90.9 to 108.6% of recovery. Samples of concentration of up to 1500.0 ng.mL^{-1} diluted showed accuracy and precision also in this range. The limits of detection and quantification were estimated with EURACHEM guide, and were obtained 0.5 ng.mL^{-1} and 4.0 ng.mL^{-1}, respectively. Cross-Talk studies, Carry-Over and specificity showed no significant interference in the procedure. **Conclusion:** The procedure developed for quantification of THC-COOH in urine specimens is simple and showed good precision and accuracy. The procedure also is sensitive enough to evaluate the use of marijuana.

FO 10- STABILITY OF ETHANOL IN REFRIGERATED POSTMORTEM BLOOD - A PRELIMINARY STUDY

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Introduction: Identification of proper storage conditions has been a matter of concern for most forensic toxicology laboratories, and the stability of ethanol and other abused drugs has been extensively studied. However, in several "real cases", data on temperature, time and stored conditions are not satisfactory or not available. In general, the analysis of the biological material is done as soon as it is received, however analysis of evidence custody may be requested several months after receipt. **Objective**: The present study aimed to investigate long-term stability of ethanol in 44 postmortem blood samples collected in the state of Rio de Janeiro, Brazil, as well as evaluate the presence of other volatile compounds and alterations in physical aspect of samples (odor, coagulation, color). **Methods**: Specimens of blood were collected in non-sterile glass tubes containing sodium fluoride as preservative (1-2%, w/v). The time, temperature, and storage between collection and sending were not controlled by the forensic toxicology laboratory. As soon as received, the samples were stored at 2-8 ◦C (in commercial refrigerators). Ethanol was quantified routinely by headspace gas chromatography flame-ionization detection (CG-FID). The analyte concentration was determined initially and at two different time intervals (6 months or 12 moths). **Results**: In 6 months study, no sample presented significant produces of ethanol. Losses in 3 samples of group A (initial concentration above 6 dg/L) were considered to be of forensic relevance. After 6 months, acetaldehyde, formaldehyde, propyl alcohol and isopropyl alcohol were detected in two of these samples. In 12 moths study, one sample presented significant produce of ethanol and area increment of acetaldehyde and propanol. Losses in 5 samples of group A (initial concentration above 6 dg/L) were considered to be of forensic relevance. After 12 months, the two most common volatile compounds detected were propanol (4 samples) and acetaldehyde (5 samples). Changes in concentration did not seem to have a clear correlation with alteration of physical aspect (color, odor). **Conclusions**: These results indicate the feasibility of preparing materials and validity of reported data if the ethanol concentration is above 6 dg/L, or if no other volatiles is detected, after six months storage. Losses of ethanol might be correlated with produces of other volatile compounds not detected initially. However, further studies are warranted to confirm this hypothesis. The authors suggest caution in interpretation of analytical results as the cases are too diverse to have a specific analytical scheme.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

FO 11- ETHANOL ANALYSES AND *CAUSA MORTIS* IN THE STATE OF RIO DE JANEIRO, BRAZIL, 2014.

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Introduction: Many difficulties may be encountered when performing forensic toxicological tests, especially if laboratory facilities are limited. In the state of Rio de Janeiro, generally, all cases are routinely subjected to a complete necropsy, and toxicological analyses are performed whenever requested by medical examiners. These analyses are based on information from the Requisition form and case history when provided. **Objective**: The present study aimed to investigate the relationship between ethanol concentration in postmortem blood samples and *causa mortis* or other relevant information. The included cases (449) were selected from all forensic necropsies that were performed during 2014 in the state of Rio de Janeiro (Brazil). The samples were distributed according to *causa mortis* information related to detection of ethanol in concentration above 4 dg/L in whole blood. The age and the sex of these groups were also reported. **Results**: The study shows that, from 449 cases included, information described in forms were accident / hard-hitting action (124 cases), fire weapon projectile (FWP) (69), hospitalized patients (49) , disease (33), natural death (19), suicide (12), sharp-cutting action (10), carbonization (5), and sudden death (2). In 110 cases, the request form was not completed and *causa mortis* or other relevant information was not described. In cases that disease, FWP (fire weapon projectile), hospitalized patients, and, suicide were reported; ethanol positive samples (ethanol above 4 dg/L) were, 5%, 7%, 10%, and 13%, respectively. The study revealed that positive ethanol cases were higher when described the *causa mortis* were traffic accident/hard-hitting action (44%), and sharp-cutting action (50%). Of 380 ethanol analysis requests, 48 were impaired (sample condition not appropriated to analysis). In most cases deceased sex were male (FWP, 90%; accident, 83%; disease, 67%). When age was evaluated, group A (18-30 years) represented 77%, 41%, and 21% of these cases, respectively. **Conclusion**: The results confirm that ethanol positive cases were higher in traffic accident / hard-hitting. Ethanol analysis in the disease and FWP cases seams not be forensic relevant. In FWP, ethanol positive cases were higher in the male and group A age (18-30) when compared to female or (> 30). In addition, the study shows that access to relevant information (*causa mortis*, medical, social and occupational history, and the results of other investigations) may be useful to avoid unnecessary toxicology analysis.

Acknowledgements: This work was supported by the Institute of Legal Medicine Afrânio Peixoto IMLAP, Rio de Janeiro Civil Police. The deceased whose samples were used in this study are mostly thanked for.

FO 12- AMPHETAMINE, COCAINE AND CANNABINOIDS USE AMONG TRUCK DRIVERS ON THE ROADS OF SAO PAULO STATE, BRAZIL

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Introduction: Each year an estimated 1.2 million people are killed in road crashes worldwide and as many as 50 million are injured. In Brazil, there were more than 44,000 traffic-related deaths in 2014. The use of psychoactive substances while driving is considered a major contributing factor for the occurrence of these accidents. In addition to alcohol, the most used illicit drugs in our country are amphetamines, cocaine and cannabis. Amphetamines and cocaine are used by some truck drivers to combat the detrimental effects of fatigue in cases they have to drive for many hours in a row. Therefore, it is necessary to better understand this problem in order to help authorities for the implementation of public policies related to drug use by truck drivers in Brazil. **Aims:** The objective was to evaluate the prevalence of amphetamine, cocaine and cannabis use among truck drivers in the highways of the State of Sao Paulo through toxicological analysis in urine and to correlate the results with sociodemographic and occupational data. **Methods:** Data collection was carried out between 2008 and 2012. This observational cross-sectional study included 1,316 drivers who were randomly stopped by the police (not under investigation for *suspected DUI). The volunteers* provided a urine sample after signing a consent form and answered a questionnaire with sociodemographic and occupational data. The urine samples were analyzed by immunoassay and gas chromatography-mass spectrometry. **Results and Discussion:** Of the total samples collected, 7.8% (n = 103) were positive for one or more tested drugs and/or its metabolites, with 3.4% positive for amphetamine, 2.8% for cocaine and 1.1% for cannabinoids. The remaining 0.5% corresponded to cases with more than one drug. The three drugs were found during almost the whole studied period, except in 2008. Toxicological findings were distributed differently according to some variables. Age and marital status were associated with psychostimulant drug use, while the employment type and period, ethnicity and education were not. Travel length and night resting period were also associated with stimulant drug use. Daytime resting period, travel length period, driving time without rest, number of occupants and freight content did not correlate significantly with stimulant drug use. However, the association between alcohol use (reported by truck drivers) and use of stimulant substances was found. **Conclusion:** The results indicate that the use of psychoactive substances by truck drivers is common and the use of stimulant drugs is associated with age, marital status, distance traveled and night resting period.

Key words: Amphetamine; Cocaine; Cannabis; Street Drugs; Accidents, Traffic.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FO 13- EXTERNAL CONTAMINATION ASSESSMENT USING AUTHENTIC HAIR SAMPLES FROM CRACK COCAINE USERS

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Introduction: The primary aim of this study is to estimate the amount of drug deposited externally on hair samples from a large number of established crack cocaine users. **Objective**: To corroborate the use of the wash residue data in the interpretation of hair analysis when external contamination is suspected. **Material and Methods:** A set of 138 hair samples were selected from crack cocaine users that were sent to laboratory for analysis. All samples were washed before extraction and analysed for AEME, benzoylecgonine, cocaine, cocaethylene and norcocaine by LC-MS/MS. The criterion for selection of samples for the study was the detection in the hair samples of the crack cocaine marker, AEME, above cutoff levels. The methanolic wash residues of the sub-set samples were analysed subsequently by LC-MS/MS. **Results and Discussion:** AEME, benzoylecgonine and cocaine were detected in all hair samples; cocaethylene and norcocaine were detected in 74 and 104 hair samples, respectively. Cocaine data in the wash residues (Wash), in the hair samples (Hair) were 0.5 ng/mg and 24.7 ng/mg, respectively. Five cases showed wash residue to hair ratios above 0.1: one sample was at 0.11, three at 0.13 and one at 0.36, all five hair cocaine levels were less than 12 ng/mg. All hair samples with cocaine levels above 15 ng/mg showed the wash residue to hair ratio below 0.07. Using authentic hair samples from confirmed crack cocaine users and analysing their wash residues, we showed the extent of cocaine externally deposited in 105 out of 138 hair samples. Interestingly, all five cases in which the wash residue to hair cocaine was above 0.1 (one case was 0.36) had low hair cocaine levels. In contrast, the ratios from samples with high levels of hair cocaine produced a maximum ratio wash residue to hair of 0.07. The levels of cocaine detected in the wash residue are a combination of the extent of external contamination from own drug use, which is affected by personal hygiene, and from the small quantities extracted during the wash process. It is not possible to measure the amount of drug that is extracted by the wash procedure, but a small quantity is assumed to leach out during the wash process. We showed that by comparing the levels of cocaine in the wash residue with the levels detected in the hair sample, external contamination is differentiated from drug use in most cases. These results confirm the importance of an adequate wash protocol to remove externally deposited cocaine coupled with the analysis of the wash residue when required. This is important when low levels of cocaine are detected with no metabolite from true low cocaine use or when true external contamination is suspected.

FO 14- DETECTION OF ADULTERANTS IN HAIR SAMPLES AS A DIAGNOSTIC AID IN CLINICAL PRACTICE

ANDRAUS M.[1]; TSANACLIS L.[1, 2]; RIBEIRO M.[3]; LARANJEIRA R.[3]; PISANESCHI C.[1]; SALVADORI M.[1]; WICKS J.[1, 2]

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Introduction: Inert agents are the commonest of a range of compounds used as adulterants in crack cocaine, although active pharmaceutical ingredients are also present. Levamisole, a veterinary antihelminthic drug and phenacetin, an analgesic, which is banned in many countries for its carcinogenic properties, are two of the most common adulterants of street cocaine. **Objective:** The aim of this pilot study is to assess the levels of phenacetin and levamisole in hair samples of crack/cocaine users. **Materials and Methods:** 172 hair samples were sent to the laboratory to be analysed for AEME, benzoylecgonine, cocaine, cocaethylene, norcocaine, levamisole and phenacetin by LC-MS/MS. **Results and Discussion:** Data for the analytes in the cocaine group, levamisole and phenacetin in the hair samples were as follows: cocaine was detected in 170 hair samples and levels ranged from 0.2 ng/mg to 166.2 ng/mg, median 7.7 ng/mg. AEME, benzoylecgonine, cocaethylene, norcocaine were also detected in 148, 153, 95, and 96 hair samples, respectively. Levamisole was detected in 34 hair samples (range: 0.2 ng/mg to 14.6 ng/mg; median: 0.4 ng/mg) and phenacetin was detected in 75 hair samples (range: 0.2 ng/mg to 50.6 ng/mg; median: 1.5 ng/mg). Data for the ratio of Levamisone to cocaine levels and of phenacetin to cocaine grouped according to levels of cocaine detected in the hair samples and showed that higher ratios were present in the lowest cocaine level groups for both adulterants. Chronic use of levamisole is associated with muscle pain, headache, fever, insomnia, dizziness and convulsions and phenacetin is associated with nephrotoxicity. Because of their toxicity there is particular clinical concern about their chronic use. The detection of levamisole and phenacetin in hair samples is an important tool for clinicians to diagnose the cause of symptoms in patients not related to crack cocaine use, but to the toxicity associated to these adulterants. The finding that the levels of both adulterants are inversely proportional to the levels of cocaine in hair suggests that users might be taking larger doses of the adulterants and smaller doses of cocaine, than they might believe they are.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FO 15- ANALYSIS OF DRUGS SEIZED IN THE AREA COVERED BY THE 3RD SCIENTIFIC POLICE NUCLEUS OF THE STATE OF PARAÍBA IN THE RANGE OF 2012 TO 2014

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The drug abuse is a problem of health and public security, since, besides causing chemical dependency and reduction in quality of life, still has social consequences, being strongly correlated to increased criminality and violence. It is known that the registry of drug seizures by police directly reflects the potential consumption and trafficking in a region. Based on this context, this study aimed to determine the percentual frequency of the drugs analyzed in the *3rd Scientific Police Nucleus of the State of Paraíba*, in the range of 2012 to 2014. It was a quantitative, descriptive and retrospective study, which had as object the drugs that were seized and sent for analysis at the Forensic Laboratory of the *3rd Scientific Police Nucleus of the State of Paraíba*. Data collection occurred through the institution's registry books, and the variables were analyzed through the computer program *Statistical Package for Social Sciences* (SPSS 20.0). In the three years studied, there were 1,248 seizures of different types of drugs, noting an increase of 26.6% between 2012 and 2014. The marijuana had the highest frequency among the seized drugs, registering a percentage of 54.79% in 2012, 57.07% in 2013 and 63.24% in 2014, confirming the studies that point it as the most used illicit drug worldwide. In relation to the samples containing cocaine, it was observed a gradual decrease in the percentage of seizures, which corresponded to 42.82% in 2012, 40.66% in 2013 and 31.72% in 2014, following the global trend of reduction of cocaine consumption. Also, among the drugs seized in the years of 2012, 2013 and 2014, the organic solvents were present at 1.86%, 1.26% and 3.53% of cases, respectively, generating an increase of 89.79% between years of 2012 and 2014. In addition, there was observed a growing profile in the number of seizures of different types of tablets, with an increase of 137.74% between the years 2012 and 2014, with seizures mainly in prison units. Given the results, it is observed that marijuana and cocaine are still the most representative substances in seizures that occurred in the region. However, as has already been noted, this profile tends to diversify over the years due to the arrival of new illegal drugs in other parts of the country, especially synthetic drugs, so should the experts and organs of repression be prepared for changes in current situation.

Keywords: Drugs; Marijuana; Cocaine.

FO 16- CHARACTERIZATION OF THE TRAFFIC ACCIDENTS WITH FATAL VICTIMS RELATED TO USE OF ALCOHOL

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Considered as problems of health and public safety, traffic accidents are directly related to high morbidity and mortality rates in Brazil, having the consumption of alcoholic beverages as one of the main factors responsible. This study aims to characterize traffic accidents with fatal victims who were under the influence of alcohol that occurred in the area covered by the *3rd Scientific Police Nucleus of the State of Paraíba*, Brazil, in the years of 2012 and 2013. It is about a quantitative, descriptive and cross-sectional study. The sample consisted of 275 fatal victims of traffic accidents, being 142 in 2012 and 133 in 2013, whose bodies were examined at the *Nucleus of Legal Medicine and Odontology of Campina Grande city* (NUMOL-CG) and presented positive results for alcohol in the toxicological exam. As material for data collection was used the Necropsy Reports and their Technical Reports of Alcoholic Dosage. The blood alcohol concentration tests were performed on *Instrumental Analysis Unit* at the *Institute of Scientific Police of the state of Paraiba* (IPC-PB), by the Gas Chromatography method with the separation technique of "headspace". All variables were subjected to analysis by the program *Statistical Package for Social Sciences* (SPSS 20.0). Of the victims in both years, 93.0% were male, predominating in 2012 the age group of 22-25 years (22.0%) and in 2013 the range of 18-21 years (13.5%). In 2012 accidents occurred mainly in November (14.0%), October (13.0%) and February (13.0%), while in 2013 there was a higher frequency of accidents in May (15.5 %) and July (10.5%). Regarding the location it was observed that, in most cases, accidents happened outside the city of Campina Grande - PB, which represented 83.0% of the cases in 2012 and 78.9% in 2013. In 2012, the main transport involved in the accidents was the motorcycle (61.0%), a percentage that has increased in 2013 (76.0%). In relation to blood alcohol levels, the range between 1.5 to 2.0 g / L of blood was the one most frequent in the years of 2012 (23.0%) and 2013 (22.6%). The range from 0.01 to 0.5 g / L was also significant in 2012 (19.0%) and 2013 (9.0%), indicating that the nonexistence of any alcohol concentration in blood is the more safe way of driving. Considering that the male young adult is the main subject involved in fatal accidents with individuals under the influence of alcohol, it is suggested that programs and campaigns to reduce the number of injuries and deaths from external causes have to be intensified, emphasizing this specific group. Still, it is proposed the preparation of more effective public policies as well as more rigorous monitoring of the aplication of these actions.

Keywords: Blood alcohol concentration; Traffic-accidents; Public Security.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FO 17- OPTIMIZATION OF LIQUID-PHASE MICROEXTRACTION (LPME) TECHNIQUE FOR WHOLE BLOOD IN FORENSIC TOXICOLOGY

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Introduction: The extraction of drugs from their biological matrix is a crucial step in forensic toxicology. Not only are the compounds of interest separated from the interferences but also the lifetime of the instrument and chromatographic column are extended. However, conventional liquid-liquid or solid-phase extractions are unable to answer some issues encountered with forensic samples. Recently a new type of extraction known as solvent-phase microextraction has been used for a series of applications. Microextraction techniques work with volumes in the range of microlitres, which allows for multiple analyses of limited-amount samples such as infants or decomposed bodies. These techniques are also environmental-friendly since they dramatically minimize the use of hazardous solvents. **Objective:** The main aim was to access one particular technique known as liquid-phase microextraction (LPME). This project was focused on the optimization of this technique in order to apply it, in the future, to routine casework using whole blood as the matrix of choice as it continues to be considered the gold standard in forensic toxicology. **Materials and Methods:** A set of model compounds was used in the method evaluation, including amitriptyline, cocaine, methadone and tramadol using methadone-d_9 as internal standard. Extracts were analysed by gas chromatography-mass spectrometry on a GC-MS Scion Bruker instrument. This technique consisted of coating the wall of a hollow fibre with an interface solvent and filling the lumen with an acceptor phase. The parameters tested included: solvent used, stirring mode, time of extraction, pH of solution and dilution factor. **Results and Discussion:** Recovery values of around 70% were obtained, with a maximum of 89% in the case of tramadol and a minimum of 45% for cocaine. Therefore, a combination of satisfactory recoveries and mild conditions for each of the individual parameters studied resulted from: using dihexyl ether as the interface solvent of choice within the pores of the hollow fibre, ultrasonication for 15 minutes with addition of 0.7 mL of 0.01mol.L^{-1} NaOH to 0.3 mL of whole blood. **Conclusion:** This method has shown to be capable of extracting four different basic drugs from whole blood, producing clean extracts with minimum use of hazardous solvents and overall extraction time. Hence, it can be said that it is now ready for further validation and subsequent use for laboratory routine analysis.

FO 18- SIDE EFFECTS IN THE CONSUMPTION OF ANABOLIC ANDROGENIC STEROIDS AND DIETARY SUPLEMENTS

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Introduction: A large number of adolescents and young adults use dietary supplements (DS) and abuse anabolic androgenic steroids (AASs) to improve their physical fitness and appearance. In Brazil, according to the Brazilian Association of Nutritional Products Companies (Abenutri) it is estimated that 2% of the population (4 million) are supplements consumers. The misuse involves enhance the user's appearance and sporting performance. This trend is alarming, and a phenomenon that may contribute to the serious side effects, such as the abnormal kidney function and renal dysfunction due to the use of these unaccompanied products. The use of these products is commonly associated with cases of renal failure and calcification, poisoning and hypervitaminosis according to the Brazilian Society of Nephrology (SBN). **Aim:** Investigate the relationship between the consumption of AASs and DS and alterations in the parameters of renal function. **Methods:** A total of 40 volunteers (20 professional athletes soccer and 20 bodybuilders) participated. The method involves three stages: (1) Administration of a self-completion questionnaire; (2) Assessment of biochemical parameters of renal function of volunteers' creatinine, urea and uric acid and (3) Toxicological urinalysis. The urine samples were analyzed by Liquid-Liquid Extraction (LLE) in basic and acid conditions and GC-MS. **Results:** A total of 40 questionnaires were collected from volunteers', which 20 affirmed the consumption of AASs and DS. Average levels of total and free testosterone of the participants whose consumed AASs showed an increase of 20 to 60% compared to reference values. Analysis of volunteers' results of biochemical parameters showed an elevation in serum creatinine, urea and uric acid levels. LLE method in urine presented extraction efficiencies, good recovery and selectivity to analyze low concentrations of the COOHTHC, amphetamine, benzoylecgonine, methamphetamine and MDMA. Results of urinalysis confirmed the self-reports in questionnaires. **Conclusion:** The toxicological urinalysis showed that the stimulants drugs are the most used among the group that affirmed the combination AASs and DS consume. The health risks are substantial, and the AASs and DS consume can cause synergistic side effects increasing the risks of the nephropathy, damage or kidney disease silent, eventually leads to the formation of kidney stones until severe acute renal failure. The numbers of users can increase exponentially in the coming years, given the potential biological adverse effects of AASs and DS use, the consequences for these users and their immediate surroundings, the importance of prevention is obvious. The regulated market of the products should be intensifying, and people should seek professional guidance.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

FO 19- VITREOUS HUMOUR: REAL-TIME ANALYSIS OF OPIATES USING EXACTIVE FTMS

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Introduction: The term "opiates" refers to substances derived from the opium poppy (*Papaver somniferum*), such as morphine and codeine, and also includes some semi-synthetic substances like heroin or oxycodone.[1] Vitreous humour is composed of 99% water, with the remaining 1% made up of sugar, salts and proteins. It is also subjected to less contamination and bacterial degradation due to the protected environment inside the eye, which makes it available for analysis in cases in which blood samples have already been degraded.[2] Exactive ESI FTMS technique, provides a one-to-one molecular formula *m/z* relationship due to its ultra high resolution and mass accuracy, being the right choice for exact mass determination.[3] Integrating biochemical, toxicological and forensic scopes, the aim of this work was therefore to develop a method based on Exactive Fourier Transform Mass Spectrometry (Exactive FTMS) for real-time analysis of opiates and their metabolites in vitreous humour non-decomposing bodies. **Method:** The vitreous humor samples were obtained from 10 bodies attended by team of forensic medicine from the West zone from the Medico-Legal Institute of Police Technical Scientific Superintendence — SPTC of São Paulo, Brazil. This study was approved by the Research Ethics Committee of the School of Medical Sciences at the University of Campinas – CEP/FCM (Ethics Protocol Approval number *1270/2010*) as well as by Scientific Committee of the Institute of Forensic Medicine of Technical Police Scientific Superintendence – DTD-IML (Ethics Protocols Approval numbers *09/11*, *687/2012* and *736/2012*). The samples were composed of 100 µL of vitreous humour (diluted 100x) and added 50 µL of 150 ng/ml opiates (heroin and morphine) and their metabolites solutions both diluted in 1 mL of acetonitrile with 100 mM Ammonium Acetate. The mass/charge (*m/z*) of opiates were monitored in ESI positive mode by a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). These drugs and their metabolites were identified by comparison with the mass spectra of compounds standard. **Results and discussion**: In the present work, opiates and their metabolites were detected in *post-mortem* vitreous humour analyzed. Thus, for its analytical stability and sterility for a large number of days after death, vitreous humour is a good sample for toxicological analyzes. Finally, the proposed method based on real-time analysis of opiates by Exactive FTMS proved to be satisfactory, constitutes an important tool for "*fingerprinting*" in the *post-mortem* toxicological analysis, which is essential to the toxicology and forensic pathology routine.

[1] Journal of Analytical Toxicology 2015, 39, 203–212.
[2] *Journal of Analytical Toxicology* 2012, 36, 162-170.
[3] Spectrometry Reviews, 1998, 17, 1-35.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

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A | R | T
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VT 01- ASSESSING THE EFFICACY OF AQUEOUS GARLIC EXTRACT AGAINST SUB-LETHAL CYANIDE TOXICITY USING RIGHTING REFLEX RECOVERY PARADIGM IN MICE

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Introduction: Cyanide is widely distributed in the ecosystem and has been associated with toxic effects in humans and animals. The onset of cyanide toxicity is sudden! Hence, needs dynamic and immediate treatment to overcome the toxic effects. **Objective:** To assess the efficacy of aqueous garlic extract as antidote against sub-lethal cyanide toxicity using righting reflex recovery paradigm in mice. **Materials and Methods:** Male mice weighting about 20-40 grams were divided into 30 treatment groups. The righting reflex recovery paradigm was based upon signs of acute cyanide toxicity including disturbance of neuromuscular coordination viz. the righting reflex. So, times required for *recovery* of this righting reflex with increasing doses of cyanide were measured to develop a dose-response curve. A cyanide dose that disrupted this righting reflex for approximately 1 hour with minimal deaths was then selected. Prior to each experiment, three or more mice were treated with cyanide at this dose to verify recovery time. Using this model, the aqueous garlic extract (AGE) at different doses was tested *pre-* and *post-cyanide* either singly or in adjunction with sodium nitrite (SN) or sodium thiosulfate (STS). These antidotes were administered either intraperitoneally (i.p) or orally. The efficacy was measured based upon reduction in righting reflex recovery time. **Results and Discussion:** When AGE was given singly at 250, 500 or 750 mg/kg i.p. or orally, *pre-* or *post-cyanide*, righting reflex recovery time was significantly reduced ($P<0.05$) at increasing dose of AGE. When SN was used at 20 mg/kg i.p., *pre-* and *post-cyanide*, righting reflex recovery time was 44.16±0.477and 49.33±0.494 min, respectively. STS at 600 mg/kg *pre-* and *post-cyanide* reduced the righting reflex recovery time to 17.33±0.333 and 25.5±0.341 min, respectively. On the other hand, when SN and STS were used in combination, the righting reflex recovery times were 16.0±0.447 and 22.83±0.600 min *pre-* and *post-cyanide*, respectively. AGE (at 750 mg/kg) in combination with SN, the righting reflex recovery times were reduced to 15.50±0.447 and 21.66±1.032 min, *pre-* and post-*cyanide*, respectively. Combination of AGE and STS *pre-* and *post-cyanide*, decreased the righting reflex recovery time to 8.5±0.42 and 18.5±0.494 min, respectively. On comparing the righting reflex recovery time of mice treated with AGE+SN or AGE+STS, it was significantly reduced ($P<0.05$) when used AGE+STS. None of the mice receiving either of antidotes exhibited gross changes in vital organs. Contrarily, mice injected with cyanide alone demonstrated pin point hemorrhages on heart, liver and lungs. **Conclusions:** Aqueous garlic extract either singly or in combination with STS is efficacious to treat sub-lethal cyanide toxicity in mice.

VT 02- THE HEPATOPROTECTIVE EFFECTS OF WHOLE PLANT EXTRACTS OF *OCIMUM BASILICUM* IN EXPERIMENTALLY INDUCED LIVER DAMAGE IN RATS

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The liver plays a central role in the metabolism of various endogenous and exogenous substances. This therefore exposes the liver to all kinds of insults produced by many materials including drugs, plants, domestic and industrial chemicals. More than 900 drugs have been implicated with liver injury. There are few medications mentioned to have ameliorating effects on liver damage. However some medicinal plants are reported to have hepatoprotective effects on liver damage. *Ocimum basilicum* of the plant family Lamiaceae is widely cultivated as a nourishing food and traditionally used as an antispasmodic, aromatic, digestive, carminative, stomachic, antinephrotoxic and tonic agent. This study was conducted to evaluate the antioxidant and hepatoprotective activity of *Ocimum basilicum* whole plant extracts using acetaminophen induced liver damage in rats as an experimental procedure using silymarin (100mg/kg) as a reference agent. Dried homogenized whole plant of *O. basilicum* was subjected to gradient extraction using chloroform, diethyl ether, ethyl acetate and methanol and the extracts obtained from these tested for hepatoprotective activity by pre-treating groups rats with different dosages for 7days before inducing liver damage with acetaminophen at 750mg/kg on the eight day. Liver function test was conducted by determining levels of serum liver enzymes as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, blood urea nitrogen (BUN), total protein and histological studies. The solvent extract with the highest bioactivity was further subject to vacuum liquid chromatography (VLC) and the fractions assayed for their antioxidant properties using DPPH radical scavenging and the ferric reducing antioxidant power assay (FRAP) procedures with ascorbic acid as standard. Results showed that all the solvent extracts significantly reduced ($P<0.05$) the levels of the serum enzymes AST, ALT, and ALP with significant reduction ($P<0.05$) also in BUN, total protein and total bilirubin when compared with silymarin. However the highest reduction was seen with the methanol extract with the experimental rats manifesting only few histopathological changes in their liver compared with those of the rats pre-treated with the other solvents. The VLC fractions from the methanolic extract produced significant DPPH and FRAP activities when compared with that of ascorbic acid. The results of the studies suggest that the plant *Osimum basilicum* may have hepatoprotective with antioxidant activities and may support the folkloric medicinal use of the plant in handling some diseases.

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A | R | T
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VT 03- EFFECTS OF *IPOMOEA CARNEA* IN PLACENTAL TISSUE. EVALUATION IN RODENTS AND RUMINANTS

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Introduction. *Ipomoea carnea* (*I.carnea*) is a plant that is widely distributed in northeastern Brazil and in other tropical countries. Intoxication of livestock that chronically ingest this plant has been reported in several countries, being goats the main affected species. *I.carnea* contains the indolizidine alkaloid, swainsonine, as well as toxic calystegines. Related plants worldwide are *Astragalus* and *Oxytropis* species (so-called locoweeds); however, these two genera do not contain the additional toxic calystegines. swainsonine cause cellular accumulation of oligosaccharides, due to inhibition of several important enzymes, resulting in cellular vacuolization and cell death in different organs. It is well known that *I.carnea* ingestion during pregnancy leads to changes in fetal development in rats and goats; however, little is known about the effects of the *I.carnea* in placenta. **Objective**. The aim of the present study was evaluate the effects of I.carnea in the placental tissue of rats and goats. **Material e Methods**. Pregnant rats of experimental group were treated orally by gavage, once a day from gestation day (GD) 6 to GD19, with 7,0 g/kg of *I.carnea* aqueous fraction. Control group received only tap water by gavage. At the end of pregnancy (GD20) cesarean section was performed and placental tissue was collected for histophatological and histochemical (lectins) evaluation. Pregnant goats of experimental group were treated which *I.carnea* fresh leaves at dose of 5 g/kg/day, since gestation day 35 until parturition. Control animals received no experimental treatment. Placental tissue was collected at parturition for histophatological and histochemical (lectins) evaluation. **Results and Discussion**. Placental tissue from experimental rats showed labirinth zone thickening and reduction of the junctional zone thickness, however the vacuolar degeneration was not observed in this organ, although when performed the lectin-histochemistry technique, it was possible to observe the accumulation of some sugars in some cells located at several regions of the placenta. Placental tissue from experimental goats showed cytoplasm vacuolization in columnar epithelium of chorioallantoic membrane, lectin-histochemistry technique revealed higher markup for the SWGA and WGA lectins suggesting that the vacuolated cells contain b-(1-4)-N-acetyl-glucosamine, and N-acetyl-neuramic acid in vacuoles, one of the main carbohydrates accumulated in this toxicosis. **Conclusions**. The results clearly revealed that the placental tissue is also target of toxic action of the toxic active ingredients present in the *I.carnea*. Probably, changes in fetal development observed in these two animal species exposed to *I.carnea* are also a consequence of the injury in the placental tissue that inevitably causes losses to the developing fetus.

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A | R | T
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MUTAGENESIS AND CARCINOGENESIS

A | R | T
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MC 01- EVALUATION OF CULTURED HUMAN MELANOCYTES EXPOSED TO CARBARYL AND SOLAR RADIATION

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Carbaryl (1-naphthyl-methylcarbamate), a broad spectrum insecticide, has recently been associated with the development of cutaneous melanoma in an epidemiological cohort study with U.S. farm workers also exposed to ultraviolet radiation, which is known to be the main etiologic factor for skin carcinogenesis. Although comprehensive and well designed, the epidemiological study is not sufficient to characterize the direct contribution of the insecticide and solar radiation in melanomagenesis. Several studies have explored the synergistic effect of certain chemicals with UV radiation, increasing its deleterious effects on the skin, possibly contributing to tumor development. We hypothesized that Carbaryl exposure associated with UV solar radiation may induce increased melanocyte lesions. This study aims to characterize human melanocytes after individual or combined exposure to Carbaryl (100uM) and solar radiation (375 mJ/ cm^2). In a microarray analysis, Carbaryl, but not solar radiation, induced an important oxidative stress response, evidenced by the upregulation of antioxidant genes, such as Hemeoxygenase-1 (HMOX1), and downregulation of MiTF, the main regulator of melanocytic activity; results were confirmed by qRT-PCR. Moreover, both Carbaryl and solar UV induced a gene response that suggests DNA damage and cell cycle alteration. The expression of genes in these categories, such as p21 and BRCA1/2, was notably more intense in the combined treatment group in an additive manner and in fact, flow cytometry assays demonstrated cell cycle arrest in S phase, reduced apoptosis induction and faster induction of CPD lesions in this experimental group. Our data suggests that carbaryl is genotoxic to human melanocytes, especially when associated with solar radiation.

Keywords: Carbaryl, melanocytes, microarray, solar radiation, melanomagenesis.

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MC 02- PHYTOCHEMICAL SCREENING, ANTIOXIDANT, CYTOTOXIC AND MUTAGENIC ACTIVITIES OF *Bidens pilosa* L. EXTRACT OBTAINED FROM FOUR LOCALITIES OF ESPÍRITO SANTO-BRAZIL.

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Introduction: *Bidens pilosa* L. is a weed traditionally used for the treatment of liver injury, jaundice, diabetes, laryngitis, among others disorders. Some studies showed that their ingestion can promote the proliferation of tumor cells, though their cytotoxic and mutagenic effects are not known. It is a plant of therapeutic interest to be rich in secondary metabolites useful to human health, which can vary considerably with environmental factors, affecting its therapeutic action. **Objective:** Evaluate the mutagenic and cytotoxic activity of *B. pilosa* hydroalcoholic extract from four different regions and differentiates them about the aspects phytochemicals and antioxidants. **Materials and methods:** Samples were collected in four localities of Espírito Santo (Brazil): Afonso Cláudio, Barra de São Francisco, Cariacica e Muniz Freire. The hydroalcoholic extract was obtained by maceration with 70% alcohol. The mutagenicity and cytotoxicity tests were performed in bone marrow cells of *Swiss* albino mice (*Mus musculus*), according Krishna and Hayashi (2000). The animals were divided into 14 experimental groups (n=6; males) and a single dose of extract was administered orally (100, 200 and 300mg/kg body weight b.w, gavage). Animals of positive control group were injected intraperitoneally with cyclophosphamide (100mg/kg b.w), whereas, animals of negative control group were treated with saline solution 0,9% (gavage). The phytochemical analyzes were performed by means of reactions for preliminary detection of some groups of constituents of secondary metabolites. Seven different concentrations of the extract (1000, 500, 250, 125, 62.5, 31.25, 15.62µg/mL) were subjected to the antioxidant assay by DPPH (0,3mM) method. The statistical analysis was performed by ANOVA and Tukey test ($p<0.05$). **Results and discussion:** The hydroalcoholic extract of *B. pilosa* showed no mutagenicity and cytotoxicity in all evaluated concentrations. However, the phytochemical analysis showed significant differences in content of secondary metabolites. The phytochemicals results showed the presence of phenolic, tannins, flavonoids and coumarins and absence of alkaloids in all extracts analyzed. In addition, there was presence of naphthoquinones and saponins only extract obtained from Cariacica. Such differences in the chemical composition reflected in significant differences in antioxidant activity. The free-radical scavenging activity was dose-dependent. Cariacica and Afonso Cláudio extracts showed comparable values to the standard ascorbic acid. **Conclusion:** This study concluded that the hydroalcoholic extract of *B. pilosa* showed no cytotoxicity and mutagenicity in the evaluated concentrations and presented antioxidant activity.

References: G. Krishna & M. Hayashi., *Mutat. Res.* 1-2,455 (2000).

A | R | T
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Volume 1 / Suppl. 1 / 2015

MC 03- EFFECTS OF FERTILIZATION ON MUTAGENICITY, CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF THE HYDROALCOHOLIC EXTRACT OF *Bidens pilosa* L.

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Introdution: *Bidens pilosa* L. is widely used in humans as therapeutic medicine to treat several diseases such as liver injury. It contains chemical compounds which present antiploriferative, cytotoxic and carcinogenic properties. The chemical composition of secondary metabolites is determined by genetic and environmental factors. External influences, such as different types of crops, can lead to significant changes in production of secondary metabolites and consequently, the therapeutic action. **Objective:** Assess the mutagenic, cytotoxic and antioxidant effects of hydroalcoholic extract *of B. pilosa* cultivated under different conditions. **Materials and methods:** The specie was cultivated in three fertilization conditions: chemical (NPK), organic (cattle manure) and without fertilization (control). To evaluate the mutagenic activity, 66 animals were randomly divided into eleven experimental groups (n=6, males): positive control (cyclophosphamide 100mg/kg body weight b.w., i.p.), negative control (NaCl 0,9%, gavage) and hydroalcoholic extract of *B. pilosa* (100, 200 and 300mg/kg b.w., gavage). For all doses, micronucleated polychromatic erythrocytes (MNPCE) frequency was evaluated at 24 hours after treatment. Cytotoxicity was evaluated by the polychromatic and normochromatic erythrocytes ratio (PCE/PCE+NCE). Their *in vitro* antioxidant activities were assessed by the DPPH and ABTS scavenging methods with microplate assays, using an ELISA reader. Seven different concentrations of the extract (1000, 500, 250, 125, 62.5, 31.25, 15.62 µg/mL) were tested. The statistical analysis was performed by ANOVA and Tukey test ($p<0.05$). ***Results and discussion:*** In the mutagenicity assessment, all doses of hydroalcoholic extract of *B. pilosa* without fertilization (control) resulted in no significant increase of MNPCE when compared to the negative control. In contrast, significant differences were seen for animals treated with concentrations of 100 and 200mg/kg, with the plants cultivated with chemical fertilizer and organic fertilizer, respectively, indicating a mutagenic effect on the cells. The cytotoxicity was not evident in all treatments with *B. pilosa.* In the antioxidant assay, the extract with plants without fertilization exhibits better performance in both tests (DPPH and ABTS). The results of Costa et al. (2008) indicated genotoxic potential of *B. pilosa* infusion and decoction *in vitro*, on HTC cells, while Hong et al. (2011) found no mutagenic action in the *in vitro* Ames test. **Conclusion:** *B. pilosa* did induce mutagenicity, when compared with negative control. Thus, both the cultivation as the dose suggests caution in the phytotherapeutic use of this plant.

References:

1. R.J. COSTA *et al*., *J Ethnopharmacol*. 1:118, 2008.
2. C-E. HONG *et al*., *T.J.P.R.* 2:153, 2011.

MC 04- MUTAGENICITY OF IPRIFLAVONE IN MICE WITH OSTEOPOROSIS INDUCED BY DEXAMETHASONE

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Introduction: The ipriflavone is a semi-synthetic isoflavone, which has been widely used in medicine and food supplement, and is recommended for the prevention of osteoporosis in postmenopausal women. **Objective:** The objective of this study was to evaluate the cytotoxic and mutagenic activity of ipriflavone through the micronucleus assay in bone marrow cells of healthy (males and females) and osteoporotic (females) mice. **Materials and methods:** This study was approved by Research Ethical Committee on Animal Use of Universidade Federal do Espírito Santo (CEUA/UFES, 013/2008). 90 *Swiss* albino mice (*Mus musculus*), 30 males and 60 females, aged 6–8 weeks, 30 g body weight (b.w.), were housed in plastic cages under controlled light and temperature conditions, water and food *ad libitum*. The osteoporosis was induced in female mice by intramuscular injection of dexamethasone sodium phosphate (7mg/kg b.w.), once a week up for four consecutive weeks. Therefore, the osteoporotic mice aged 10-12 weeks at the beginning of treatment. The remaining animals were maintained in pre-experimental conditions for four weeks. The animals were divided into six groups, (5 males and healthy females/group, plus 5 osteoporotic females/ipriflavone group): negative control (NaCl 0,9%, gavage, g.); positive control (cyclophosphamide, 50 mg/kg b.w., intraperitoneal injection); vehicle control (DMSO, 0.01 mL/g b.w., g.); sub clinical, clinical, and supra clinical ipriflavone doses (1.71, 8.57, 42.85 mg/kg b.w.,respectively, single dose for five days, g.). The mutagenicity effect was evaluated by analyzing 2000 polychromatic erythrocytes (PCEs) per animal, scoring the micronucleated polychromatic erythrocytes (MNPCE) frequency. The cytotoxic effect was evaluated by the ratio of PCE, in 400 erythrocytes. The statistical analysis was performed by t-test ($p < 0.05$), using Assistat 7.6 beta software. **Results and discussion:** The dose 1,71 mg/Kg was more cytotoxic and mutagenic for both males and healthy females. The dose 8,57 mg/Kg showed more cytotoxicity in osteoporotic females than in the healthy ones. Furthermore, statistical analysis showed no mutagenicity between healthy and osteoporotic females. The dose 42.85 mg/Kg was more cytotoxic for males when compared to the healthy and osteoporotic females. This high cytotoxicity may have caused the low MNPCE frequency observed in males. The difference between sexes may be due to the metabolism and hormonal activity. The mutagenicity and cytotoxicity may be related to pro-oxidant effect of some flavonoids. **Conclusion:** The clinical dose of ipriflavone in osteoporosis treatment demonstrated similar to healthy females, confirming the its use as an alternative hormone replacement.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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MC 05- MICRONUCLEUS TEST AND PREVENTION OF CERVICAL CANCER DEVELOPMENT

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Introduction: Human papillomavirus (HPV) is the major cause of cervical cancer development, the second most common cancer in Brazilian women. Early diagnosis is necessary to develop clinical procedure to control and minimize viral infection and prevent cancer development. However early diagnosis is relatively expensive. **Objective:** The aim of this study was realize a survey literature to associate micronucleus (MN) frequency in HPV diagnosed patients with disease severity. **Material and methods:** Literature search was performed on NCBI, MEDLINE, SciELO and Portal de Periodic CAPES. The search was conducted using the Boolean Operator AND and the terms "micronucleus" and "HPV", general terms chosen to make possible evaluate the greatest number of articles published in area. The abstract of the articles obtained was read, after the articles that addressed the subject of this survey were selected and was considerate for this literature survey only research articles published on the last ten years. The data about age, country of origin of the studied population, patients number and principal results was collected and organized on a table. Review articles and other kind of scientific manuscripts were not considered in this literature survey. **Results and discussion:** The search obtained 153 scientific articles and after the analysis of its abstracts four Research Articles were selected. These articles were published between 2008 -2015 and all of them had case-control experiment design. Three of these studies were realized in Brazil and just one was realized in Mexico. The average age of patients varied between 11-43.9 years, and patients positives for HPV infection were aged between 11-34.2 years. All analyzed studies suggest direct relationship between MN frequency increased and HPV disease, however only one study evaluated the association between MN frequency, level of cervical lesions and viral load in patients with HPV. This study established an association between viral load, high level of cervical lesions and increased of MN frequency. In other hand, another study associating the increased of MN frequency and risk factors for cervical cancer development concluded that the prevalence of micronuclei in cervical cells was higher in patients with one or more risk factors for cancer uterine than in patients without risk factors. These studies suggest that the increase of genomic damage is associated to HPV disease severity and indicate that increases in MN frequency could stay associated to cervical cancer development. **Conclusion:** Studies associating MN frequency and HPV disease severity are rare and more studies needs to be realized to validate the MN test, a low-cost test, as technique in the control and prophylaxis of cervical cancer.

MC 06- ASSOCIATION BETWEEN GENOMIC DAMAGE AND POLYMORPHISMS OF GSTM1 AND GSTT1 GENES IN CHRONIC ALCOHOLICS THE MUNICIPALITY OF GOIÂNIA

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Ethanol is one of the main drugs for humanity. In Brazil this consumption intensified, since colonial times, with the production of rum. Currently, alcohol consumption is an important public health problem in Brazil and in the world, with a risk factor of great impact for mortality and disability of people. Brazil has been prominent on the world stage by the average per capita consumption of absolute alcohol per year, with 40% above average. The main health entities in the world, governmental and non-governmental, highlight that chronic ethanol consumption causes physical and psychological dependence, in addition to increasing the risk of liver disease and some cancers. This drug is a substance absorbed quickly by the body and metabolized primarily in the liver, the cytosolic pathway, through the participation of enzymes such as aldehyde dehydrogenase and alcohol dehydrogenase. In alcoholics, due to chronic consumption of this substance, the main metabolic pathway is the microsomal (MEOS) producing intermediate metabolites, such as reactive oxygen species (ROS), increasing the concentration of these radicals in their bodies. Both the cytosolic pathway as the route microsomal there is the production of acetaldehyde, which as well as the ROS is a toxic substance for its ability to bind to organic molecules, such as DNA and proteins, causing harm. In this context, the present study was designed to investigate whether there was a relationship between these DNA damage, assessed by the comet assay with the polymorphism of GSTM1 and GSTT1. Our research is a case-control study including 105 adults, 66 alcoholics (case) and 39 non-drinkers (control). Peripheral blood samples were collected for evaluation of GSTM1 polymorphism and GSTT1, for real time PCR and for assessing genotoxic damage measured by the comet assay, this assay is a sensitive genotoxicity study method that enables quantify DNA damage. Polymorphism of GSTM1 and GSTT1 genes did not correlate with increased damage to the genome of the alcoholics. The understanding of other genotypes of susceptibility and the evaluation of mutagenicity testing is an important step in understanding the damage increase mechanisms in the human genome with possible implications for organic disorders.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

MC 07- CYTOTOXIC EFFECTS OF 4'-HYDROXYCHALCONES, TERPENOYD-LIKE BIS-CHALCONES AND SULFOMIDE CHALCONES DERIVATIVES TOWARDS CANCER CELL LINES

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Chalcones are alfa,beta-unsatured ketones founded in plants and they can also be synthesized by Claisen-Schmidt reaction. Several substituents in different positions on the core structure of chalcones can determine different biological activities as well as specificity of action [1]. In addition, sulfonamide chalcones, hydroxychalcones and bischalcones derivatives have received significant attention due to their antitumor properties [2,3]. The aim of present study was to evaluate the antitumor activity of hydroxychalcones, terpenoid-like bischalcones and novel sulfonamide nitrochalcones against three tumor cell lines, OVCAR-8 (breast), HCT-116 (colon) and (SF-295) central nervous system by MTT method. The chalcones derivatives were synthesized by Claisen Schmidt condensation using substituted acetophenones or ionones and benzaldehydes under basic media and products were characterized by NMR and IR. In the MTT assay, cells were exposed to 25 μg/mL of chalcones for 48h. After exposure, MTT solution was added and incubated for 4 h. The microplate was centrifuged for 10 minutes at 800 rpm and the supernatant was removed. Formed formazan crystals were solubilized in DMSO and the absorbance was measured by spectrophotometry at 595 nm. The IC50 values were determined by non-linear regression using GraphPad Prism 4.0 software. All 4'-hydroxychalcones and bischalcones tested showed significant cellular inhibition greater than or equal to 75% in at least two tumor cell lines, whereas only one sulfonamide chalcone (2,5-Dichloro-N- {3 - [(2E) -3 - (4-nitrophenyl) prop-2-enoyl] phenyl} benzenesulfonamide) showed a strong inhibition. Bischalcones with nitro (-NO_2) groups were the most potent citotoxicic agents among the tested compounds. The bischalcone 11 with 4-NO_2 groups presented the lowest IC50, 3.99 μM, in SF-295 cell line, while the 4'-hidroxychalcone and sulfonamide chalcone with 4-NO_2 groups presented IC50 of 19.89 μM and 72.67 μM, respectively, in the same cell line. The bischalcone 12 with 3-NO_2 presented the lowest IC50 value of 4.33 μM in OVCAR-8 cell line and bischalcone 14 with 2-NO_2 was the most citotoxic compound with IC50 of 2.70 μM in HCT-116 cells. Therefore, it is speculated that the higher cytotoxic effect of bischalcones compared to other chalcones is due to the presence of two alfa,beta-unsaturations in its molecular structure. In conclusion, the study demonstrates that the terpenoid-type bischalcones are promising candidates for prototype drugs because they had more potent cytotoxic profiles than the analogs, 4'-hydroxychalcones and sulfonamide derivatives, in the tested tumor cell lines.

References:
1. R.G. Damazio *et al*., *Eur. J. Med. Chem*. **45**, 1332 (2010).
2. S.A. Lee *et al*., *Hepatology* **49**, 1316 (2009).
3. E. Winter *et al., J. Med. Chem*. **57**, 2930 (2014).

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MC 08- GENOTOXICITY, CYTOTOXICITY AND MODULATORY EVALUATION OF CHALCONE (*E*)-3-(4-METHOXYPHENYL)-1-PHENYL-2-PROPEN-1-ONE (CA) USING THE MOUSE BONE MARROW MICRONUCLEUS TEST AND THE SALMONELLA/ MICROSOME ASSAY.

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Chalcones are precursors in flavonoids biosynthesis and constitute a large part of the total daily intake of natural polyphenolics. Moreover, chalcones have attracted the interest of the scientific community due to a wide variety of pharmacological effects related to them. We have recently reported the synthesis of chalcone (*E*)-3-(4-methoxyphenyl)-1-phenyl-2-propen-1-one (CA) as cytotoxic agent towards cell lines (1). However, the reports of therapeutical use of flavonoids are widely conflicting and the knowledge regarding the genotoxic and antigenotoxic effects of these compounds, including chalcones, remains limited. The aim of present study was to evaluate the genotoxic, cytotoxic, and protective effects of chalcone CA using the *Salmonella typhimurium* reverse mutation assay (Ames test) and the mouse bone marrow micronucleus test (MBMMT). For the evaluation of genotoxic activity by MBMMT at times of 24h and 48h, different doses of CA (5, 10 and 20 mg/kg body weight – b.w.) were intraperitoneally administered to groups of five animals for each treatment. In the Ames test, the *Salmonella typhimurium* strains (TA97a, TA98 and TA100) were treated with different doses of CA (0.5, 1, 5, 10 and 20, μg/plate) For the antigenotoxicity evaluation, the same doses of CA employed in the genotoxic evaluation were co-treated with their respective positive controls that were mitomycin C (MMC) in MBMMT and sodium azide (SA), 4-nitroquinoline1-oxide (4NQO) in Ames test. In the Ames test, CA inhibited mutations induced by frameshift mutagen and base pair substitution mutagen. CA, at the dose of 20 μg/plate, exhibited potent antimutagenic effects with inhibition of 81, 84 and 47% in TA97a, TA98 and TA100, respectively. The doses of CA did not increase the number of revertant colonies in either tester strain in the mutagenic assay. In relation to MBMMT, the number of micronucleated polychromatic erythrocytes increased significantly ($p < 0.05$) in rats exposed to CA after 24 h and 48 h, except at lower dose of 5 mg/Kg b.w. of CA after 24 h of exposure. All tested doses (5, 10, and 20 mg/kg b.w.) of CA showed non-citotoxicity effect. With regard to antimutagenicity evaluation after 24 h, CA were able to strongly decrease the MMC-induced MN level (32 ± 1.67, $p < 0.05$) with inhibition of 83.1%, 95.1% and 93.0% at 5, 10 and 20 mg/Kg b.w, respectively. In conclusion, CA presented moderate genotoxicity, but strong protective effects as antimutagenic, antigenotoxic and anticytotoxic agent under the experimental conditions applied in this study. However, more studies are required to better understand the protective action of CA.

References:
S. D. Ramalho et al., *Chem. Biodivers*. **10**, 1999 (2006).

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

MC 09- PROTECTIVE EFFECT OF SELECTED SOUTH AFRICAN PLANTS AGAINST THE GENOTOXICITY OF THREE ENVIRONMENTAL MUTAGENS

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Introduction: Epidemiological studies indicate that many cancers are dependent on multiple mutational etiologies as well as inherited mutator phenotypes. These mutations may include a single point nucleotide exchange, deletion, amplification, translocation, chromosomal rearrangement, methylation or other events that can lead to the activation of oncogenes and inactivation of tumor suppressor genes. The search for inhibitors of mutagenesis may therefore be useful as a tool to discover preventive agents that could be used to reduce the risk arising from exposure to various environmental mutagens by affecting various stages of cancer development. [1, 2] **Objective**: The aim of the study was to find plants endemic to South Africa with potential preventive effect against the genotoxic effects of three environmental mutagens namely; the directly acting 4NQO, MMC mutagens and indirectly acting aflatoxin B_1. **Materials and Methods:** Methanolic extracts from more than 100 plant species were evaluated for their antimutagenic effect against the three environmental mutagen-induced mutagenicity using the *in vitro* Ames (TA98 and TA100), Vitotox and comet assays. **Results and Discussion:** The results showed that none of the extracts tested in the assays were found to induce mutation. Extracts from members of the Asteraceae, Annonanceae, Leguminosea, Guttifereae and Proteaceae exhibited promising antigenotoxic activity in Ames test and/or Vitotox assay. Among these, extracts from three *Helichrysum* species exhibited excellent antimutagenic activities in the Ames and Vitotox assays while extracts from *Anonna senegalensis*, *Acacaia polycantha* and *Protea nitida* had significant antimutagenic activity in the Ames test. **Conclusions:** Plant extracts that exhibited significant antimutagenic activity are good candidates for further *in vitro* and *in vivo* investigation in the search for antigenotoxic compounds that hinder the mutagenicity of environmental mutagens, which can be used as parent compounds for animal feed additives and/or nutriceuticals for human use.

References:

J.E. Trosko & B.L. Upham, *Mutagenesis*. **20**, 81 (2005).
L. Verschaeve & J. van Staden, *J Ethnopharmacol*. **119**, 575 (2008).

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MC 10- SODIUM BUTYRATE REDUCES NUCLEAR p53 AND CRM1 EXPORTATION IN LIVER CANCER CELLS

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Introduction: Cancer prevention is the most promising strategy for reducing both cancer incidence and cancer-related mortality. One promising approach to cancer prevention is an active intervention with agents that are expected to suppress or attenuate the initial phases of carcinogenesis. In previous studies, we demonstrated a potent chemopreventive effect of tributyrin, a butyric acid prodrug, on experimental hepatocarcinogenesis. This chemopreventive effect of tributyrin has been linked to normalization of the subcellular localization of p53 protein, characterized by its increase in the nuclei[1,2]. The nuclear localization of p53 is important to its tumor suppressor activity, and can be modulated by nuclear exportin proteins such as CRM1 (ubiquitous transport receptor chromosome maintenance protein 1)[3]. **Objective:** The goal of the present study was to investigate the underlying effects of butyrate on the mechanism of p53 exportation by CRM1 *in vitro* using different liver cancer cell lines treated with sodium butyrate. **Materials and Methods:** Rat hepatoma-derived JM1 cells and, human hepatocarcinoma PLC/PRF/5 (p53 R249S) and Hep3B (null p53) cell lines were cultured until 75% confluence, and then treated with sodium butyrate for 48 hrs. Cytoplasmic and nuclear protein fractions of cells were obtained, and aliquots containing equal quantities of proteins were separated by SDS–polyacrylamide gel electrophoresis and transferred to PVDF membranes. Membranes were probed with primary antibodies against p53, CRM1, β-actin and lamin B1. Total protein extract of JM1 cells were p53 immunoprecipitated and subjected to SDS-PAGE and immunoblotting analysis for CRM1 and p53. Results are presented as mean ± S.D. Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test for pair-wise comparisons. When appropriate, Student's t-test was used. P-values < 0.05 were considered significant. **Results and Discussion:** Our results show that treatment with sodium butyrate increased ($P<0.05$) the levels of p53 and CRM1 in the nucleus, and decreased their levels ($P<0.05$) in cytoplasm. Moreover, sodium butyrate inhibited the binding of CRM1 to p53. **Conclusion:** The results demonstrate that chemopreventive activity of sodium butyrate and its prodrug tributyrin may be attributed to the inhibition of nuclear export of p53 and CRM1 and subsequent induction of apoptosis.

References:

1. Kuroiwa-Trzmielina *et al.,* Int J Cancer. **124**, 2520-7 (2009).
2. De Conti et al., J Nutr Biochem. **23**,860-6 (2012).
3.Singer et al., Mol Cell. **48**,799-810 (2012).

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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MC11- *IN VITRO* CYTOTOXICITY ASSAYS IN HUMAN CELLS FOLLOWING EXPOSURE TO FOOD SUPPLEMENT SYNEPHRINE

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Introduction: Synephrine (*p*-synephrine) is a protoalkaloid derived from the phenylethylamines group that is naturally found in the immature fruit or peel of *Citrus aurantium* (bitter orange). This substance is widely used in food supplements, which aims weight loss as well as improvement of sports performance; however, data concerning its risk assessment are still not found. **Objetive:** The aim of this study was to evaluate the possible cytotoxic activity of synephrine in human cell lines *in vitro*, and thus give further data concerning the toxicological profile of this compound. **Materials and Methods:** Two tests of cytotoxicity were performed, MTT and Neutral Red (NR) uptake assays in HepG2 cells (human hepatocellular carcinoma cell derived with metabolization system), Caco-2 cells (human intestinal adenocarcinoma cells) and primary cultures of normal gastric cells which were subjected to the treatments with ten different concentrations (25 - 5000 µM) for a period of 24 hours, as well as negative (PBS); solvent (Perchloric Acid 5%) and positive (Methyl Methanesulfonate; MMS/300 µM) control groups. **Results:** In MTT assays, only HepG2 cells exposed to the concentration of 5000 µM effectively decreased the cell viability by 21%, while no cytotoxic effects were seen in Caco-2 and primary gastric cells cultures. In NR assays, no citotoxic effects were observed in primary gastric and HepG2 cells exposed to the several concentrations of synephrine; however, in Caco-2 cells, concentrations up to 800 µM induce disturbances in the cell viability, which gives piece of evidence that high concentrations of synephrine may induce cytotoxicity. **Conclusion:** Therefore, it can be concluded, so far, that data obtained from MTT and NR assays indicate that synephrine, a substance used as a food supplement with slimming purposes, has no effects on the viability of human cells *in vitro* at low concentrations.

Key words: synephrine; cytotoxicity; human cell lines *in vitro*; risk assessment

Financial Support: CNPq; FAPESP (Proc. 2014/20344-9); CAPES.

MC 12- METABOLIC WARNING: DISRUPTION OF ENERGETIC METABOLITES PRECEDES CELL TRANSFORMATION BY B[*a*]P EXPOSURE

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Introduction: Connections between metabolic disruption and cellular transformation have been verified in some genetic diseases and studies considering a possible role of the Warburg effect on tumor development resurrected. Although genotoxic and epigenotoxic events are well accepted to play a role in tumor development, there is no data regarding simultaneous assessment of metabolic disruption and these events after carcinogen exposure. Benzo[*a*]pyrene (B[*a*]P) is a widely studied carcinogen that is widespread in the environment. It is accepted that its carcinogenicity is due to reactive intermediates that induce DNA damage and subsequent mutations. Therefore, B[*a*]P was chosen as a suitable carcinogen for this study. **Objectives:** We evaluated here an association between cell metabolic disruption, epigenetic changes, genotoxicity, and cell transformation induced by B[*a*]P in the normal human bronchial epithelial cell line BEAS-2B. **Material and Methods**: BEAS-2B cells were incubated with B[*a*]P (0.1-1 µM) for 1-168h. Cytotoxicity was assessed by CVD. Metabolic activity was determined by XTT, SDH activity and glucose consumption assays. Analyses of cell cycle, DNA and membrane integrity were done by flow cytometry. Global DNA methylation and hydroxymethylation, DNA mutagenic lesions, and 14 intracellular metabolites were quantified by HPLC-ESI-MS/MS. Transformation and mutation frequency were monitored by the soft agar and HPRT assays. Statistical analyses were performed by Kruskal–Wallis test. **Results**: B[*a*]P was absorbed by the cells and induced cell growth arrest by 72-168h in a dose-dependent manner. Membrane damage, DNA fragmentation, G0-G1 and G2-M cell cycle arrest were revealed after 168h incubation with 0.5 and 1.0 µM. All tested concentrations induced a boost in mitochondrial enzyme activity. Time-dependent increase of intracellular ADP, fumarate, glutamine, glutamate, malate, lactate, NADH, NADPH, succinate, pyruvate ($p<0.05$), and glucose consumption (168h, $p=0.0027$) were observed in cells exposed to 0.5 and 1 µM, maybe as an adaptive response to preserve ATP levels. DNA repair has been verified by a dose-dependent increase of DNA lesions in the culture medium. Additionally, no mutagenic event was observed in the HPRT assay. Exposure for 168h led to a global DNA hypermethylation. Tumor induction was observed after cell growth in soft agar, with evident DNA global hypomethylation and hyperhydroxymethylation. **Conclusion:** Metabolic disruption by B[*a*]P may promote cell transformation via epigenetic changes of gene expression, leading to a tumor phenotype. To date no study had shown an association between these events resulting from B[*a*]P or other carcinogen exposure.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 13- CHEMOPROTECTIVE EFFECT OF DIPHENYL DITELLURIDE ON DOXORUBICIN INDUCED TOXICITY IN MAMMALIAN CELLS

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Introduction: Doxorubicin (DXR) is one of the commonly used chemotherapeutic agents in the treatment of hematological malignancies. Anthracycline-induced cardiotoxicity is believed to be related to the generation of reactive oxygen species (ROS) by at least two mechanisms: enzymatic reduction of the quinone with subsequent redox cycling and/or formation of an iron-anthracycline complex capable of intramolecular reduction and redox cycling. Some chemotherapeutic approaches have proposed the use of antioxidants to minimized cytotoxicity and the damage induced in normal tissues by antitumor agents that produce free radicals. The reactivity of organotellurium compounds is characterized by high nucleophilicity and antioxidant potential. Diphenyl ditelluride (DPDT) is a compound with antioxidant and antigenotoxic potential. However, the beneficial properties occur in a limited concentration range due to a bimodal nature of this agent. **Objectives:** The aim of this study was to evaluate the effect of low DPDT concentrations on DXR-induced toxicity and genotoxicity in Chinese hamster fibroblasts (V79), as well as in human fibroblasts proficient (MRC5) and deficient in NER (XPD). **Methodology:** For this purpose, the cell lines MRC5, V79 and XPD were treated with doxorubicin in the presence or absence of DPDT pre-treatment. Measurement of cell viability was performed using MTT assay. The DNA damage induced by DXR was studied in the comet assay and modified comet assay including incubation with the enzymes formamidopyrimidine DNA glycosylase (Fpg), that is specific for oxidized purines, and endonuclease III (Endo III) that recognizes mainly oxidized pyrimidines. The intracellular ROS levels were visualized by fluorescence microscope following incubation with 2′-7′-dichloro-dihydrofluorescein diacetate. **Results:** DXR at concentration of 0.6 µg/mL induced genotoxicity, increase in the Fpg- and Endo III sensitive sites and elevated intracellular ROS levels after 3h treatment. The effect of a range of DPDT concentrations (10 nM, 50 nM and 100 nM) on DXR induced cytotoxicity and genotoxicity at same conditions was evaluated. The concentration of 10 nM DPDT decreased DXR-induced genotoxicity and ROS formation. Concentrations above 100 nM DPDT enhanced the cytotoxic effects of DXR. **Conclusions:** Our results showed that low DPDT concentrations exhibit chemopreventive effect on DXR-induced DNA damage without decreasing its cytotoxicity in mammalian cells. This finding suggests that DPDT can be useful for preventing the Anthracycline-induced genotoxic damage in normal tissues.

Financial support: CNPq/CAPES.

MC 14- CYTOTOXICITY SCREENING OF 2-CARBOXY-2-HEPTANE-INDOLIN-3-ONE IN HUMAN CELL LINES AND PROLIFERATION, CELL CYCLE AND MEMBRANE INTEGRITY OF MCF-7 CELL LINE

BARANOSKI A.[1]; BIAZI B.I.[1]; ZANETTI T.A.[1]; CORVELONI A.C.[1]; OLIVEIRA, M. T.[1]; OLIVEIRA A.G.[2]; SIMIONATO A.S.[2]; NAVARRO M.O.P.[2]; FILHO G. A.[2]; MANTOVANI M.S.[1]

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Introduction: Compounds that have as its targets the pathways involved in tumor cells growth and survival are promising candidates as anticancer agents. In this context, derivatives of indolinones have shown receptor inhibition of protein tyrosine-kinases, inhibiting the proliferation of various cell cancer lines. The 2-carboxy-2-heptane-indolin-3-one (Indolin-3-one) derivative compound is a possibly novel indolinone, which is extracted from *Pseudomonas aeruginosa*. This compound showed anti-proliferative and cytotoxic effects on HepG2 cells and downregulated the expression of genes involved in carcinogenesis process, demonstrating its potential. **Objective:** Perform a cytotoxicity screening on tumor cell lines and a non-tumor cell line and investigate the proliferation, cell cycle and membrane integrity of MCF-7 cells treated with Indolin-3-one. **Materials and Methods:** The cytotoxicity screening was performed using the MTT assay in tumor cell lines HepG2/C3A, MCF-7, HT-29, DU-145 and NCI-H460 and non-tumor cell line HB4a after 24 and 48 hours of treatment using dosages of 0.125; 0.25; 0.5; 1; 5; 10 and 20 µM of Indolin-3-one. Cell count for proliferation analysis was performed by MUSE Cell Analyzer, and cell cycle and membranes integrity in MCF-7 cells were evaluated by flow cytometry using doses of 0.25; 1; 5; 20 µM of Indolin-3-one at 24, 48 and 72 hours of treatments. **Results and Discussion:** The screening of cell lines has shown that the compound has higher potency of action after 48 hours of treatment, particularly in the MCF-7 line. It has also demonstrated no cytotoxic effect on the non-tumor cell line for the tested concentrations, which demonstrates a possible selective action for tumor lines. The cell count analysis for proliferation in MCF-7 cells demonstrated that cell number was reduced to the concentration of 20 µM in 48 and 72 hours, without affecting the integrity of membranes for any tested dose or time. The cell cycle analysis demonstrated an increase of cells in G1 phase to the concentrations of 5 and 20 µM, at 24, 48 and 72 hours as well to the concentration of 1 µM in 72 hours. This shows that the cytotoxicity in MTT assay, seems to be due the decreased proliferation, and the G1 phase arrest may be a consequence of growth factors inhibition since others indolinone derivatives induce arrest at the G1 phase by this mechanism. **Conclusions:** A higher action on tumor cells, mainly in MCF-7, and no cytotoxicity in non-tumor cells at tested concentrations shows a possible selective action of this molecule. The inhibitions of the cell proliferation, while maintaining membrane integrity and G1 phase increase demonstrates the potential of this compound, as well as guide for later studies.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 15- KINETICS OF PROLIFERATION TUMOR CELLS AND NOT TUMOR TREATED WITH 2-CARBOXY-2-HEPTANE-INDOLIN-3-ONE

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Introduction: New compounds which exhibit inhibition of tumor cell growth and does not interfere with the non-tumor cell-cycle are very interesting for future treatments of cancers. In this context, the compound 2-carboxy-2-heptane-indolin-3-one (indolin-3-one) which is extracted from *Pseudomonas aeruginosa* has cytotoxic effects on MCF-7 cells (tumor) without any effect on cells HB4a (non-tumor) demonstrating selective cytotoxicity in MTT assay. **Objective:** This study aimed to evaluate the kinetics of proliferation in MCF-7 cells (tumor - breast adenocarcinoma) and HB4a (non-tumor breast cells) treated with indolin-3-one. **Materials and Methods:** The cell proliferation kinetics was performed using the RTCA SP xCELLigence apparatus in breast tumor cell line MCF-7, and in non-tumor cell line HB4a, of breast, using doses of 1; 5; 10; 20 and 40 μM of indolin-3-one. The proliferation kinetics was monitored for more of 72 hours after treatment. **Results and Discussion:** The proliferation kinetics show that the Indolin-3-one cause delay proliferation in MCF-7 (tumor cell) in a dose dependent manner, being more evident in doses of 20 and 40 μM after 48 hours. In cell line HB4a (non-tumor) there was no interference in proliferation, even after over 72 hours of treatment. Inhibition of proliferation found for tumor cells has been demonstrated in MTT assay for MCF-7 cell, as no inhibition in HB4a. In MCF-7 was also shown that the inhibition is due to the increase of cells in G1 phase, in addition, other derivatives by indolinone have been described as inhibitors of tumor growth by inhibiting cell growth factors. **Conclusions:** The growth retardation in tumor cells demonstrates the potential of indolin-3-one as anticancer drug. The non-interference in non-tumor cell line is a result that demonstrates selectivity or greater potency and for action on tumor cells.

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MC 16- MIXTURE OF PESTICIDES METHOMYL AND CYPERMETHRIN SHOW GENOTOXIC EFFECTS IN VIVO

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Introduction: The use of pesticides in plantations increases every year. Farmers use a wide variety of pesticides, leading the population exposing to a large number of toxic agents through diet. Therefore, the use of pesticides has generated many questions about its effect on health of consumers. Within the possible harmful effects, we highlight the genotoxic and mutagenic. There are a large number of studies using tests such as the micronucleus (MN) and the comet assay (CA) to quantify damage caused by pesticides. Daily, the consumers are exposed to mixtures of pesticides; however, most studies have focused on the detrimental effects of isolated pesticides, what does not represent the human exposing. **Aim**: To evaluate the *in vivo* genotoxic and mutagenic effects of acute ingestion of the mixture of pesticides methomyl (Mt) and cypermethrin (Cp), considering doses of acceptable daily intake (ADI). **Material and Methods**: Fifty mice (*Swiss* albino) were divided in five groups. Three groups were treated with Mt and Cp mix: group A (0.0005 mg/kg Mt + 0.00125 mg/kg Cp); group B (0.005 mg/kg of Mt + 0.0125 mg/kg of Cp); and group C (0.05 mg/kg of Mt + 0.0625 mg/kg of Cp). These values were based on the values established for the Acceptable Daily Intake (ADI). The negative control (NC), received distilled water through gavage. The positive control group received a dose of cyclophosphamide (20 mg/kg) by intraperitoneal injection. After 48 hours, peripheral blood was collected by retro-orbital puncture. Blood was immediately used in MN test and CA. *ANOVA* followed by Tukey post-test was used for statistical analysis, with confidence interval of $p < 0.05$. *R software* was used for all analyzes. **Results and discussion**: In the MN test, neither treatment showed significant difference (mean A= 2.6 ± 1.26; B= 3.0 ± 1.33; NC= 3.1 ± 1.20), except for the C group (8.2 ± 2.10). In the CA evaluation, all concentrations showed significant outcomes when compared with NC, suggesting that three concentrations are genotoxic. Others studies showed that the Mt alone presents genotoxic and mutagenic effects. The Ct, however, did not demonstrate genotoxic effect. The Mt and Cp combination demonstrated both genotoxic and mutagenic effects, mainly in higher concentrations. **Conclusion**: In our current conditions, the pesticides combination demonstrated both genotoxic and mutagenic effects, even when the doses administrated were in accord to the ADI establishments and ANVISA suggestion. Exposure to mixtures of pesticides, which is closer to reality than exposure to isolated pesticides, may demonstrate genotoxic effects different for those demonstrated by isolated pesticides.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 17- ASSESSMENT OF THE MUTAGENICITY AND GENOTOXICITY IN VIVO OF DERIVED SYNTHETIC PYRAZOLE OF USNIC ACID ISOLATED FROM *Cladonia substellata*

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Introduction: The usnic acid is one of the most studied secondary metabolites of lichens because of its diverse biological activities such as antitumor, antibacterial, anti-inflammatory, antiviral, among others. Despite the great biological potential of usnic acid, its mutagenic and genotoxic activity is still unknown. In addition, the fact that usnic acid has low solubility in organic solvents and in water, and has a very high hepatotoxic potential arouses interest in structural modification of the molecule to reduce hepatotoxicity and induce more favorable solubilities without losing their biological activities, can make it an effective prototype in the treatment of diseases. **Objectives:** Evaluate the mutagenic and genotoxic activity *in vivo* of derived synthetic pyrazole of usnic acid obtained from *C. substellata.* **Materials and Methods:** The usnic acid was obtained by purification from the ether extract of *C. substellata.* The pyrazole derivative was synthesized by reaction of usnic acid with substituted phenylhydrazine and equivalent amounts of sodium bicarbonate, affording the compound 16-Acetyl-13,15-dihydroxy-3,6,14-trimethyl-1- (8-bromophenyl) -1H [1] benzofuro [9,5-f] indazole 10 (2H) -one (3B). The mutagenicity and genotoxicity *in vivo* were evaluated by the micronucleus test and comet assay, respectively. The animals were divided into four groups (n = 5): usnic acid (15mg / kg), derived synthetic pyrazole (15 mg / kg), negative control (100 uL of DMSO and PBS-phosphate buffer saline; the vehicle in which the usnic acid and the derivative was dissolved) and the positive control group, which received cyclophosphamide (micronucleus test) and hydrogen peroxide (comet assay). Statistical analysis was performed by analysis of variance using a parametric test (ANOVA) and a nonparametric test (Kruskal-Wallis), considering $p \leq 0.05$. The R 3.1.0 software was used for analysis. **Results and Discussion:** The usnic acid exhibited a high degree of purity and the synthetic approach presented satisfactory returns. Polychromatic erythrocytes with micronucleus were analyzed (MNPCE), as well as the intensity and frequency of DNA damage. According to statistical analysis, there was no significant difference when treatment groups was compared with negative control. Therefore, substances analyzed were not mutagenic nor genotoxic considering the experiment conditions. **Conclusion:** The micronucleus analysis and comet assay showed that the tested substances (pyrazole derived and usnic acid) are not mutagenic and genotoxic for the treated animals. The data presented are encouraging and broaden the horizons for new tests in order to increase knowledge about these new molecules.

MC 18- EFFECT OF SODIUM SELENITE ON HYDROGEN PEROXIDE-INDUCED CYTOTOXICITY, CELL PROLIFERATION KINETIC, CELL CYCLE AND DNA DAMAGE IN HepG2/C3A CELLS

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Introduction: Reactive oxygen species (ROS) are involved in reactions in the biological system that can initiate various pathological processes. These effects can be minimized by some compounds with antioxidant ability. The selenium element, an essential micronutrient, is highly important for human health for having antioxidant activity. Sodium selenite (SS) is an inorganic form and source of selenium for the body, however, its cytoprotective capacity and antioxidant ability remains to be clarified. **Objective:** Verify the protective effect of the SS against hydrogen peroxide (H_2O_2) in cytotoxicity, cell proliferation kinetic, cell cycle and DNA damage in human hepatocellular carcinoma cell line (HepG2/C3A). **Materials and methods:** HepG2/C3A cells were pre-treated with SS (20 and 200 nM) for 24 h, then co-incubated with H_2O_2 (300 µM) for 2 h. Then fresh medium was added with no treatments and held for 0, 22 or 94 h of cell recovery according to the performed assay. The effects of SS on the oxidative stress caused by H_2O_2 were assessed for cytotoxicity using MTT assay (22 h). Cell proliferation kinetic was evaluated using *xCELLigence* RTCA equipment (*Real-Time Cell Analyzer* – ROCHE) maintained for 94 h. The cell cycle analysis was performed by flow cytometry (propidium iodide – (PI) stained) after 22 h. Genotoxicity was evaluated by the comet assay after 2 h of treatment (SS + H_2O_2) without recovery time. All experiments were performed with three biological replicates and the statistical analysis were performed using ANOVA/Dunnet and Kruskal-Wallis/Dunn ($p<0.05$). **Results and discussion:** It was observed in MTT assay that there was significant difference between the positive control (H_2O_2) and groups treated with SS + H_2O_2, however this difference was not found in RTCA test in all the analyzed time. On cell cycle analysis, it was found that co-treated groups were equal to the positive control which showed significant reduction in G1 phase and an increase in the G2/M phase. The comet assay showed that SS has antigenotoxic activity, as there was a reduction of DNA damage in co-treated groups. In this way, the genotoxicity and cytotoxicity caused by H_2O_2 can be reduced by the presence of selenium, since the same constitutes the active center of antioxidant enzymes. The SS has an important protective role against oxidative stress produced by reactive species of oxygen which, in turn, cause damage to lipids, proteins, nucleic acids and other biomolecules, thereby inducing cell death or inhibition of cell proliferation. **Conclusion:** The results suggest that SS has cytotoxicity and genotoxicity protection against the damage caused by H_2O_2, however this effect was not shown in cell cycle and cell proliferation kinetic.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

MC 19- CYTOTOXICITY, KINETIC OF PROLIFERATION, CELL CYCLE AND APOPTOSIS IN NCI-H460 CELLS TREATED WITH HYDROGEN PEROXIDE

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Introduction: Hydrogen peroxide (H_2O_2) is an aerobic metabolic and important reactive oxygen species (ROS). When the amounts of ROS in the cells exceed its antioxidant capability, the cell can come to a condition known as oxidative stress. In this condition, the excess of these elements may have adverse effects on cell components such as lipids, proteins, nucleic acids and other biomolecules. **Objective:** Evaluate the cytotoxic potential, kinetics of cell proliferation, cell cycle and apoptosis in lung cancer non-small cells (NCI-H460) treated with different concentrations of H_2O_2. **Materials and methods:** NCI-H460 cells were exposed to H_2O_2 at concentrations of 150, 250 and 350 µM for 24 hours. The effects of H_2O_2 were measured for cytotoxicity using colorimetric MTT assay, kinetics of cell proliferation by Real-Time Cell Analyzer (RTCA), and flow cytometry were all used to analyze cell cycle, cell membrane integrity (propidium iodide) and apoptosis (Annexin V CF647/7-AAD). The statistical analyses were performed using ANOVA/Dunnet and Kruskal-Wallis/Dunn ($p<0.05$). **Results and discussion:** There was a significant decrease in the viability and cell proliferation when the cells were exposed to H_2O_2 in all concentrations, in a dose-dependent manner. Furthermore, it has been verified that membrane integrity was damaged by exposure of H_2O_2 in all concentrations. This effect can be explained by their lipid peroxidation action and damage of membrane proteins. In cell cycle analysis, a significant increase was observed in the sub-G1 phase at concentrations of 250 and 350 µM. This phase indicates the presence of DNA fragments which are a characteristic of apoptosis. Furthermore, there was a significant increase in the percentage of cells in G1 phase when treated at the highest concentration (350 µM). Since the G1 phase is the period when the gene transcription and translation, leading to synthesis of proteins necessary for DNA synthesis, it is closely linked to the proliferative state of the cell. In apoptosis analysis, it was observed a dose-dependent increase of initial and final apoptosis, confirming the data found on the cell cycle analysis. **Conclusion:** It is considered that H_2O_2 can be a good model for oxidative stress induction studies, since the same cytotoxic activity exerted with alteration of cell cycle phases and induced cell death by apoptosis in a dose dependent manner.

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MC 20- IMPACT OF AGRICULTURAL BRAZILIAN AREAS IN THE GENOME OF *Physalaemus cuvieri* (AMPHIBIA-ANURA) TADPOLES: AN *IN SITU* GENOTOXICITY APPROACH

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Introduction: Amphibians inhabiting agricultural ecosystems are constantly exposed to large amounts of pesticides that end up reaching the aquatic environment during the rains, through runoff, drainage and leaching. The indiscriminate use of these compounds can have a negative impact on amphibians that rely on water to spawn and where the majority of tadpoles of several anuran species live until metamorphosis. **Objective:** The aim of this study was to evaluate the impact of agroecosystems on genome of *Physalaemus cuvieri* tadpoles. **Material and methods:** In this context, we evaluate the potential genotoxic effects of pesticides used in agriculture, on the genome of *Physalaemus cuvieri* tadpoles (n = 184), using the alkaline comet assay, in an *in situ* approach. Therefore we quantified the extensions of genomic damage in the tadpoles sampled in agricultural areas (soybean and corn) and compared and compared with those collected in areas without any kind of agriculture activity (control group). The Subjects were then collected in the field and its cells could be tested directly. This approach allowed us to estimate the damage in these animals exposed to genotoxic agents in their natural habitat. **Results and discussion:** Our results demonstrated differences in the amount of genomic damages between tadpoles from areas under the influence of pesticides when compared to those in the control group. We found that, tadpoles collected near to soybean crops presented the highest amounts of DNA damage in all comet's parameters, being significantly higher than those found in cornfields and the control group. Our results clearly demonstrated the impact of agricultural areas with a heavy use of pesticides on the genome of exposed tadpoles, especially for those collected from soybean areas. **Conclusion:** Thus, our results indicated that the comet assay in an *in situ* strategy is a sensitive method for detecting DNA damage induced by environmental pollutants. It can also concluded that the *Physalaemus cuvieri* tadpoles may be considered as a good bioindicator of environmental quality, since it was sensitive to environmental changes and can be used in monitoring disturbed areas studies.

Keywords: amphibians; bio-indicators; exposure; genotoxicity; pesticides.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 21- EVALUATION THE POTENTIAL OF CYTOTOXIC ACTION AND MUTAGENIC *Psidium guajava* L. INFUSION ON MICE Swiss

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Introduction: *Psidium guajava* L. (guava) is a medicinal plant widely used as infusion to treat cough, diarrhea and hypertension. However, its mutagenic or genotoxic effects are not known. Many plants used in traditional medicine produce secondary metabolites, which can induce the fragmentation of chromosomes and/or damage cellular mitotic apparatus, causing the loss of pieces or whole chromosomes. **Objective**: The objective was to evaluate the cytotoxic and mutagenic potential infusion of guava leaves through the micronucleus test in bone marrow and peripheral blood of mice. **Material and Methods**: The present study was approved by the Research Ethical Committee on Animal Use of Universidade Federal do Espírito Santo (CEUA/UFES,021/2011). 48 *Swiss* albino male mice (*Mus musculus*), 6-8 weeks age and 30g weight were acclimatized (temperature 25 ±1°C; humidity 70±10%; 12-hour light-dark cycle) for one week. They were kept inside plastic cages, supplied with standard chow and water *ad libitum* throughout the study. Then, animals were randomly divided into eight groups (n=6): negative control (NaCl 0,9%), positive control (cisplatin, 0,05mg/kg body weight b.w., i.p.) and infusion of *Psidium guajava* L. var. Pedro Sato (50, 250 and 500mg/Kg b.w., gavage). The animals underwent acute treatment. The cells of bone marrow and peripheral blood were stained with Leishman. The mutagenicity effect in the bone marrow was evaluated by analyzing 2000 polychromatic erythrocytes (PCEs) per animal, scoring the micronucleated polychromatic erythrocytes (MNPCE) frequency. The cytotoxic effect was evaluated by the ratio PCE/NCE (normochromatic erythrocytes), in a total of 400 erythrocytes. Were analyzed 2000 NCEs per animal in peripheral blood sample for the presence of micronuclei. The statistical analysis was performed by ANOVA and Tukey test ($p < 0.05$), using Assistat 7.6 beta software. **Results and Discussion**: The use of infusion of *P. guajava* dried leaves did not promote mutagenic effect at the tested doses. Also, there was no cytotoxicity. The results suggest that infusion of this plant can be used as medicinal at the tested doses. The results of Teixeira et al. (2003) also indicated no mutagenicity of *P. guajava* infusion in *Wistar* rats bone marrow, human peripheral blood and *Allium cepa.* Martinez et al. (2001) also found no mutagenic action in the mice bone marrow micronucleus test. The results may suggest the *P. guajava* phytochemical compounds did not damage the DNA. **Conclusion**: This study found that the infusion of *P. guajava* var. Pedro Sato did not induce cytotoxicity and mutagenicity in the genetic material under experimental conditions.

References

1. M.J.MARTÍNEZ *et al., Rev Cub Plant Med.* 6: 56, 2001.
2.R.O.TEIXEIRA et al., *Genet Mol Biol***.** 26: 551, 2003.

MC 22- HARPAGOSIDE HAS AN ANTIPROLIFERATIVE EFFECT BY CELL CYCLE ARREST AND APOPTOTIC INDUCTION IN C3A CELLS

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Introduction: Recently, there are many researches looking for new molecules with anticancer properties by the ability to stop cell growth. Harpagoside is an iridoid glycoside found in great concentrations in *Harpagophytum procumbens*, a plant with many biological properties. Therefore, new studies are necessary to elucidate and discover the mechanisms of action of the present compounds in this plant. **Objective:** The aim of this study is to evaluate the antiproliferative effect of harpagoside on C3A cells, using the kinetics of proliferation, cell cycle and apoptotic effect analysis. **Materials e Methods**: It was used the concentrations 0, 300 and 700 µM of harpagoside. To analyze cell growth, it was applied the kinetics of proliferation during 24, 48 and 72 hours after treatments with harpagoside, and the cells were counted using Muse Cell Analyser (Merk Millipore). To verify the cell cycle, it was used a flow cytometric assay (Guava – Merk Millipore) based on the stain of the DNA with iodide propidium after 24 hours of treatments. The apoptotic effect of harpagoside was evaluated by flow cytometry (Guava – Merk Millipore) using stain with Annexin-V and 7-aad (7-Aminoactinomycin D) after 24 and 48 hours of treatments. **Results and discussion:** On the kinetics of proliferation, it was observed reduction of the cell count just after 48 and 72 hours of treatments (in both concentrations). However, in the cell cycle analysis (24 hours), there is an increase of G2/M on the 700 µM treatment. The 700 µM treatment also showed induction of apoptosis only after 48 hours of treatment. The presented data shows that harpagoside can interfere in the cell growth of this cell line, and this effect may be a consequence of the cell cycle arrest culminating with apoptosis after 48 hours of treatment. **Conclusion:** As discussed, harpagoside can act as an antiproliferative drug due its cell cycle arrest effect and induction of cell death. From this, we have a new candidate compound to be studied and explored as a new anticancer drug.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 23- ANTIMUTAGENICITY AND INDUCTION OF ANTIOXIDANT DEFENSE BY EXTRACT OF *Myrcia bella* RICH IN FLAVONOID

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Introduction: The Brazilian "cerrado" is a region that presents important source of natural products, such as *Myrcia bella* Cambess (MB, also known as "mercurinho"). MB has been used in Brazilian folk medicine for treatment of diabetes and gastrointestinal disorders; however, until now, only its hypoglycemic activity was experimentally described. **Objective:** The present study characterized biological activities of hydroalcoholic MB extract in human normal and tumoral gastric cells. **Material and Methods:** Cytotoxic effects were evaluated using MTT assay, differential staining with *acridine orange/ethidium bromide* (AO/EB) *and flow cytometry (evaluation of subG1 population). Cytostatic effects were observed in cell proliferation curves using cell counting and total protein content and cell cycle kinetics by flow cytrometry. Mutagenic and antimutagenic effects were evaluated by CBMNcyt. CM-H_2DCFDA probe was used to quantify reactive species and RT-qPCR to evaluated changes in gene expression.* **Results and Discussion:** MTT assay indicated that MB extract induced cytotoxicity in tumor cells at lower concentrations compared with normal cells. Moreover, based on *AO/EB staining* may be stated that this extract induced cell death by triggering the process of necrosis. *An antiproliferative effect was evidenced through an arrest in the G2/M phase detected by flow cytometry and by a decreasing in the nuclear division index in* CBMNcyt. *Cells treated with MB extract combined with doxorubicin (DXR) showed increase of NUBDs, which may be related to the amplification of CCND1 gene observed in RTqPCR. Antimutagenic effects were also observed in CBMNcyt and may be associated with the antioxidant activities observed using the CM-H_2DCFDA probe that detects reactive species. In summary,* our findings showed that MB extract (a) in high concentrations induced cytotoxicity and cell death by triggering the necrosis process; (b) presented antiproliferative effect associated with G2/M arrest and (c) presented antioxidant activity that could be responsible for the observed antimutagenic effects and for protective effects against gastrointestinal disorders previously described to MB. ***Conclusions:*** Although the effects observed to MB extract are not specific to normal or tumoral gastric cells, they provide a panel of biological activities for further exploration. Importantly, we provided a wide characterization of the biological effects of the MB extract as a promising candidate for the treatment of chronic diseases and showed that its antioxidant capacity could be responsible for the protective effects against gastrointestinal disorders observed in the traditional use of MB.

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MC 24- CYTOTOXIC AND GENOTOXIC EVALUATION OF CHLOROGENIC ACID, A DIETARY BIOACTIVE COMPOUND, IN ASSOCIATION WITH 5-AZACYTIDINE IN LEUKEMIC HUMAN CELLS HL -60

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Introduction: The influence of dietary bioactive compounds in genome has been evidenced in recent years and the important role of these compounds in epigenetic modulation has been also highlighted. The association of dietary bioactive compounds and medicines used in cancer therapy represents a promising alternative in order to enhance the effectiveness of the treatment and minimize adverse effects. Chlorogenic acid is a phenolic compound commonly found in fruits and vegetables, such as coffee, apples and tomatoes. Studies indicate that the chlorogenic acid inhibits DNA methylation[1], therefore it is interesting to investigate the effects of this dietary bioactive compound in association with the demethylating agent 5-azacytidine, which acts by altering the pattern of DNA methylation in the treatment of myelodysplastic syndromes and acute myeloid leukemias. **Objective:** The aim of this study was to assess the cytotoxicity and genotoxicity of chlorogenic acid associated or not with the demethylating agent 5-azacytidine in leukemic human cells HL -60. **Material and Methods:** The cytotoxicity was evaluated by the MTT Assay and Neutral Red (NR) assay and the genomic instability was determined by the comet assay. For MTT and NR assays, cells were treated with chlorogenic acid (1-250 µM) and 5-azacytidine (0.5-50 µM) or their association for 24 hours. The comet assay was performed under alkaline conditions and cells were treated with chlorogenic acid (1; 10; 100 µM), 5-azacytidine (5 µM), or their association for 4 hours. **Results and Discussion:** The viability of HL-60 cells exposed to chlorogenic acid was greater than 90% in both tests, while the treatments with 5-azacytidine at concentrations above of 20 µM and 7.5 µM were cytotoxic to cells by MTT and NR assays, respectively. The association of chlorogenic acid (1, 10 or 100 µM) with 5 -azacytidine (5 µM) did not reduce cell viability in both assays. Furthermore, no significant increase in DNA damage was observed in treatments with chlorogenic acid, 5-azacytidine or their association, when compared to negative control group. **Conclusions:** Chlorogenic acid alone or in association with 5-azacytidine did not induce cytotoxicity and genotoxicity in HL-60 cells under the conditions employed in this study. Further investigation will be carried out to establish the effects of chlorogenic acid in the pattern of DNA methylation in leukemia cell lines treated with 5-azacytidine.

References:
1. W.J. Lee & B.T. Zhu, *Carcinogenesis,* 27(2), 269 (2006)

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 25- EVALUATION OF DNA DAMAGE IN *Hypsiboas albopunctatus* (ANURA - HYLIDAE) TADPOLES: an *in situ* APPROACH

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Introduction: Amphibians represent important vertebrates in natural and agricultural ecosystems since they are included among the most important natural enemies of several agricultural pests worldwide. They possess certain characteristics rendering them a useful indicator species for measuring the effects of changes of the environment. Several reports agree in demonstrating that these vertebrates can be regarded as bioindicators of aquatic and agricultural ecosystems due not only to their sensitivity to habitat modification, but also to the presence of larvae stage. **Objectives:** The aim of this study was to evaluate the DNA damage in *Hypsiboas albopunctatus* collected in agricultural areas (exposed group) and compare to those collected in unexposed areas (control). **Material and Methods:** In this context, we evaluate the potential genotoxic effects of agroecosystems on the genome of *H. albopunctatus* tadpoles (n = 44), using the alkaline comet assay, in a case/control approach. Thus we quantified the extensions of DNA damage in the tadpoles sampled in agricultural areas (Corn crops) and compared with those collected in areas without any kind of agriculture activity (control group). The statistical analysis was performed using discriminant analysis function and Student *t* test, using the comet parameters tail length (TL), % of DNA in tail (% DNA) and Olive tail moment (OTM). **Results and discussion:** According to discriminant analysis, most of the scores associated with the exposed areas showed positive scores, in contrast to those reported in the unexposed group, which had negative scores, despite it wasn't enough to be significance different between exposed and unexposed ($p = 0.059$). We found that the OTM presented the highest contribution to discriminant function ($F = 0.954$). Regarding the comet parameters TL, % DNA and OTM, the tadpoles genome located in adjacent areas of corn crops had the highest stretches of DNA damages, being significantly higher than those found in the control group. **Conclusion:** Thus, our results demonstrate the potential negative impact of agroecosystems on genome of tadpoles exposed in their natural habitat.

MC 26- FLAVONOID HESPERIDIN PRESENTS CHEMOPROTECTION AGAINST CISPLATIN- INDUCED DNA DAMAGE IN MICE

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Introduction: Hesperidin is a natural flavonoid present in citrus fruits. Several reports have demonstrated its antioxidant properties. **Objective**: This study aimed to investigate the antimutagenic potential of flavonoid hesperidin against cisplatin-induced damages in Swiss mice, using *in vivo* micronucleus test. **Material and Methods**: The present study was approved by the Research Ethical Committee on Animal Use of Universidade Federal do Espírito Santo (CEUA/ UFES,001/2011). 60 male *Swiss* albino mice and 60 female *Swiss* albino mice (*Mus musculus*), 6-8 weeks age and 30g weight were acclimatized to the conditions (temperature 25 ±1°C; humidity 70±10%; 12-hour light-dark cycle) for one week before the experiment started. They were kept inside plastic cages, supplied with standard chow, and water *ad libitum* throughout the study. Then, animals were randomly divided into twelve experimental groups (5 males, 5 females): negative control (NaCl 0,9%, by intraperitoneal injection, i.p.); positive control (cisplatin, CddP, 0,05mg.kg^{-1} body weight b.w., i.p.), vehicle control (corn oil, c.o.); hesperidin (Sigma®) pre-treatment (100 mg.kg^{-1}, 200 mg.kg^{-1}, 400 mg.kg^{-1}c.o., b.w., gavage) during 14 days followed by CddP (0,05mg.kg^{-1}b.w., i.p.) on the 14th day. Inthe post-treatment, the same hesperidin doses was aplied after 24h of CddP (0,05mg.kg^{-1}b.w., i.p.); and a simultaneous treatment of the same hesperidin doses and CddP (0,05mg.kg^{-1}b.w., i.p.). The bone marrow cells were stained with Leishman. The antimutagenicity effect was evaluated by analyzing 2000 polychromatic erythrocytes (PCEs) per animal, scoring the micronucleated polychromatic erythrocytes (MNPCE) frequency. The cytotoxic effect was evaluated by the ratio of PCE, in a total of 400 erythrocytes. Also, it was calculated the percentage of damage reduction. The statistical analysis to compare the sex responses to the treatments was performed by Mann–Whitney test ($p < 0.05$), using Assistat 7.6 beta software. **Results and Discussion:** All the protocols presented a reduction on the MNPCE frequency compared to the CddP group. All the doses and treatments showed a significant % damage reduction, except for the simultaneous treatment 400 mg.kg^{-1} dose that had no effect on this parameter. Furthermore, there were no sex differences in all treated groups in the antimutagenicity, cytotoxicity, and % damage reduction parameters. The results suggest that the flavonoid hesperidin antioxidant effects might be involved in the chemoprotection against the DNA damages induced by the anticancer cisplatin, may be neutralizing free radicals. **Conclusion**: The hesperidin showed chemoprotective effect against cisplatin-induced DNA damages, regardless the sex.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 27- BOTRYOSPHAERAN, A NEW EXOPOLYSACCHARIDE PRODUCED BY *BOTRYOSPHAERIA RHODINA:* EVALUATION OF ITS ACTIVITY MUTAGENIC

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Introduction: β-glucans are exopolysaccharides (EPS) that constitute a structural part of fungal, yeast, oat and barley cell wall. They have received special attention due to its bioactivity and the possibility of being used as ingredients for functional foods. Botryosphaeran is an EPS that has been isolated from the culture medium of *Botryosphaeria rhodina,* easy to obtain and inexpensive. **Objective:** Having in mind these characteristics and its potential for use on a commercial scale, this study evaluated the mutagenic effect of EPS by micronucleus test in peripheral blood cells of albino Swiss young mice. **Materials and methods**: Microorganism, growth conditions and production of botryosphaeran were previously established by our research group. The study on mice was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee at Federal University of Espírito Santo. The animals were randomized into five groups each of 10 mice (5 males and 5 females) and were subjected to sub-chronic treatment (30 days) by gavage and further to euthanasia. Two groups were used respectively, as the negative (isotonic saline solution) and positive (cyclophosphamide, 100mg/Kg b.w.) controls and the other three groups received three different doses of EPS each. On the final of treatment, the positive control group received cyclophosphamide by i.p. to induce micronucleus formation. Animals of all the groups were weighed weekly. Mice peripheral blood was taken for the micronucleus tests in the times (T): T0 (zero); T15 (fifteen); T30 (thirty days) and their respective slides prepared for analysis normochromatic erythrocytes (NCE) and micronucleated (NCEMN), whereas 1000 erythrocytes. We used the Mann-Whitney test (comparison between times) and Kruskal-Wallis test (comparison between doses each time) with $p<0.05$. **Results and Discussion:** Our results showed that botryosphaeran did not exert mutagenic activity as well there was a tendency to decrease the average number of NCEMN during treatment, the three doses. In male animals, with the highest dose, at T30, there was a lower mean micronuclei number. In female, between T15 and T30, there was a significant difference in NCEMN to the higher dose (1.4 ± 1.11 and 1.0 ± 0.77). There was no significant difference in the number of NCEMN between the sexes, considering different doses and times. Miranda *et al.*[1] also found similar results for fifteen days treatment. **Conclusion:** These data help to elucidate the mechanism of action of botryosphaeran and provide subsidies for production of a novel food ingredient with potential biotechnological application, being an adjunct in the prevention or treatment of diseases.

References:
1. Int J Biol Macromol. 42: 172, 2008.

MC 28- *Mikania glomerata* HYDROALCOHOLIC EXTRACT HAS NEITHER CITOTOXIC NOR ANTIMUTAGENIC EFFECTS

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Introduction: *Mikania glomerata* (MG) is a medicinal plant extensively used in Brazil due to its antiinflammatory and bronchodilator activity. The lactone coumarin is the major compound in MG and has showed genoprotection probably in response to its antioxidant properties. However, there are few reports related to *in vivo* genotoxic effects of MG hydroalcoholic extract. **Objective:** The objective of this study was to evaluate the cytotoxic and antimutagenic activity of MG hydroalcoholic extract using micronucleus assay in mice bone marrow cells in a simultaneous treatment. **Materials and methods**: The study was approved by the Research Ethical Committee on Animal Use of the Universidade Federal do Espírito Santo (registration number 026/13). *Swiss* albino male mice (*Mus musculus*), aged 6–8 weeks, 30g body weight (b.w.), housed in plastic cages under light and temperature controlled conditions, had free access to water and food. The hydroalcoholic extract (70 ethanol: 30 water, v/v) of MG dried leaves was used in a single dose treatment based on data Lethal Dose. The animals were divided into five groups (n=6): negative control (NaCl 0,9%, gavage g); positive control (cyclophosphamide, 50 mg/kg b.w., intraperitoneal injection ip); MG treatment (107.5, 215.0 or 430.0 mg/kg b.w., g) simultaneously to cyclophosphamide (50 mg/kg b.w., ip). The antimutagenicity effect was evaluated by analyzing 2000 polychromatic erythrocytes (PCEs) per animal, scoring the micronucleated polychromatic erythrocytes (MNPCE) frequency. The cytotoxic effect was evaluated by the PCE ratio in 400 erythrocytes (PCE/(PCE+NCE)). The statistical analysis was performed by ANOVA with Tukey post-test ($p < 0.05$), using Assistat 7.6 beta software. **Results and discussion:** The PCE ratio showed no difference among negative control and simultaneous treatment groups, suggesting no cytotoxic activity ($p<0.05$). Only the group treated with 107.5 mg/Kg dose demonstrated PCEMN frequency statistically similar to positive control. Both 215.0 and 430.0 mg/Kg doses simultaneously to cyclophosphamide presented higher PCEMN frequency than the positive control ($p<0.05$), indicating that these doses increased the mutagenic damage when combined with an alkylating agent. Despite the presence of the antioxidant coumarin, other reports suggest that MG might potentiate the mutagenicity when associated with alkylating agent, as demonstrated in the present study. Thus, these results probably suggest a drug-drug interaction. **Conclusion:** In conclusion, the use extracts of MG in simultaneous treatment induces an increase in mutagenicity simultaneously to an alkylating agent, although has no cytotoxic effect.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 29- EVALUATION OF *BYRSONIMA INTERMEDIA* SPECIE IN HUMAN CELLS WITH PUTATIVE CHEMOPREVENTIVE AGENT

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Introduction: *Byrsonima intermedia,* found in an extensive area in Brazil, is extensively used by the population due to this therapeutic activity and presents gastroprotective and antidiarrheal activities. So the extract of this plant is a good candidate for inclusion as phytotherapics but studies are necessary to ensure the safety and therapeutic efficacy of this medicinal plant. **Objective:** Thus, the objective of the present study was to evaluate extract of *B. intermedia* regarding its potential antiproliferative, mutagenic and antimutagenic, genotoxic and antigenotoxic and oxidant or antioxidant effects in human hepatocarcinoma cells (HepG2) and primary gastric cell line (GAS). **Material and Methods:** MTT, LDH and neutral red assay were used to evaluate cell viability. Three non-cytotoxic concentrations (0.15; 0.6 and 2.4 µg/mL) were chosen and evaluated for cell viability using flow cytometry, proliferation curve and nuclear division index (NDI). The concentration of 0.6 µg/mL was used to assess mutagenicity and antimutagenicity using micronucleus assay (MN); genotoxicity and antigenotoxicity using the comet assay and oxidant and antioxidant activities using CM-H_2DCFDA probe. **Results and Discussion**: *B. intermedia* extract affected differently the cell viability depending on the metabolic cell state and of the biological parameter evaluated. The LDH assay was more reliable to detect non cytotoxic concentration, while in proliferation curve by counting cells and quantifying the total protein content, the reduction in viability could not be observed. *B. intermedia* also decreased the NDI in GAS cells by MN test. Using flow cytometry analysis *B. intermedia* extract decreased the number of cells in G1 phase and increased in G2 demonstrating stop to repair damage in both cell lines. MN and comet assay showed that just the inductor of DNA damage (Benzo(a)pyrene for HepG2 and doxorubicin for GAS) increased the frequency of micronuclei and the scores in comet assay. In antimutagenicity and antigenotoxicity assays, the extract of *B. intermedia* showed a protective effect on DNA damage; furthermore, this extract showed antioxidant effect in both cell lines. **Conclusion:** The protective effects in human cell lines exerced by *B. intermedia* extract indicates *B. intermedia* as chemopreventive agent and encourage futher studies to better characterize it.

Financial support: Biota-São Paulo Research Foundation (FAPESP); CAPES-DS.

MC 30- PHOTOMUTAGENICITY AND PHOTOPROTECTION OF EXTRACTS FROM THE ANTARCTIC MOSS *SANIONIA UNCINATA*

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Introduction: Under solar exposition at high levels of ultraviolet (UV) radiation, moss *Sanionia uncinata* (Hedw.) Loeske can undergo oxidative stresses. Its hydroethanolic (HE) extract has presented antioxidant activity protecting DNA against cleavage induced by reactive oxygen species, but also increasing DNA cleavage induced by Fenton-like reactions. HE also induced photoprotection against UVC radiation in NER-deficient and proficient *Escherichia coli* cells and on a frozen solution of thymine (pyrimidine dimerization). No mutagenicity has been found for HE, ethanol (EE) and methanol extracts (ME). **Objective**: Investigating the potential induction and inhibition of photomutagenicity by *Sanionia uncinata* moss that was collected in the Antarctic Peninsula. **Material and Methods**: The photo-Ames test were carried out in absolute and 70% ethanol and methanol extracts using *Salmonella typhimurium* histidine auxotrophic TA102 and TA104 strains, considering basic procedures for the *Salmonella*/microsome assay by the plate incorporation procedure, with some modifications and without exogenous metabolization. The applied UVA doses to investigate photomutagenicity and photoprotection were 6.5 J/cm^2 and 1.3 J/cm^{2}for TA102 strain and 0.24 J/cm^2 and 0.04J/cm^2 for TA104 strain. After irradiation cells were incubated in the dark at 37°C for 72 hours and histidine revertant colonies (His^+) were counted. Cytotoxicity was verified by reduction of background growth and of His^+. **Results and Discussion**: No extracts induced mutagenicity in the *Salmonella* strains after exposed to UVA, although HE induced photocytotoxicity of TA102 strain at highest concentrations after exposition of high doses of radiation. Additionally, HE and EE induced photoprotective effects in TA104 strain. No correlation between photoprotection and photocytotoxic effects was found, suggesting that His^+ decreasing is not related to the cytotoxic effect. It is interesting to point that the extracts exhibited the protective effect at very low concentrations. **Conclusion**: *S. uncinata* extracts are promising in photoprotection against the effects of UVA radiation without photomutation induction.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 31- CHEMICAL AND ANTIMUTAGENIC STUDIES OF EXTRACTS OF THE SCLERACTINIAN INVASORS CORALS *Tubastraea coccinea* AND *Tubastraea tagusensis*

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Introduction: Scleractinian corals have been ignored as natural products sources for many years, since their secondary metabolites production was not associated as a defense strategy by researches. Specifically, the genus *Tubastraea* (Scleractina, Cnidaria) has its secondary metabolites associated with ecologic activities as antifeedant and allelopathy and with pharmacology value such as toxic, antimicrobial, antiviral, antitumor and anti-inflammatory activities. This genus was probably invaded on brazilian coast, in 80's, when the first fouling record was observed on oil and gas platforms in Bacia de Campos, Rio de Janeiro state north region. The recorded species of invasive corals *T. coccinea* and *T. tagusensis* are a threat to endemic species of the region. Their invasive capacity can be attributed to the secondary metabolites production that brings a pharmacology interest. **Objective:** Investigate the chemical composition and antimutagenic activity of the metanolic extracts of *T. coccinea* and *T. tagusensis*. **Material and Methods:** The colonies were lyophilized, and extracted under ultrasonication at room temperature with methanol as solvent three times consecutives. The extracts were analyzed by high performance liquid chromatography (HPLC) and mass spectrometry techniques, and the antimutagenic potential of these samples was investigated using standards lineages of *Samonella enterica serovar* Typhimurium (*S* Typhimurium) in spot test. **Results and Discussion:** The mass spectrometry data show a peak at 255,1213 m/z with *T. coccinea* and 255,1232 m/z with *T. tagusensis* suggesting the presence of an alkaloid compound known as aplysinopsin. This compound is also found in others species of the same genus. The HPLC data reinforce this hypothesis. The TA97, TA98, TA100, TA102 and TA104 *S.* Typimurium strains were exposed to 5, 25, 50, 250 and 500 µg/plate of both extracts in the presence of 4-Nitroquinoline *N*-oxide. An antimutagenic activity was observed for TA97 at 250 and 500 µg/plate concentrations in co-treatment for both species extracts. **Conclusion:** We suggest the presence of substances with pharmacology value in *T. coccinea* and *T. tagusensis* extracts composition with an antimutagenic activity observed that may occur by an extracellular reaction.

Acknowledgements: CAPES, Faperj

MC 32- ASSESSMENT OF THE GENOTOXICITY AND ACUTE TOXICITY OF "DISPERSE VIOLET 93" DYE WITH THE ZEBRAFISH EMBRYO TEST

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Introduction: Recent studies are highlighting the relation between the chemical structure of dyes and environmental toxicity. In this context, the "Disperse Violet 93" dye was repeated identified in textile effluents[1] as one of the components responsible for the mutagenic activity of the samples by the Ames test with *Salmonella typhimurium* YG1041 strain[2]. In addition to the toxicity for the aquatic life of the rivers near the industries, other toxicological tests are important to determine the potential risk for the human consumption of these waters. **Objective:** To do the genotoxicity test with human keratinocytes, and the zebrafish embryo acute toxicity test with the "Disperse Violet 93" dye. **Material and Methods:** The genotoxicity studies were performed with the purified "Disperse Violet 93" dye using the Comet Assay with HaCaT cells based on Östling and Johanson methodology (1984), according to the changes proposed by Singh et al. (1988), and following the protocol established by Tice et al. (2000) with some adjustments. The zebrafish embryo acute toxicity test was based on the procedures and endpoints described by the OECD #236 guideline. All the tests used concentrations of the dye between 0.1 and 5,000 µg / mL. **Results and Discussion:** The zebrafish embryo acute toxicity test showed positive results above the 50 µg / mL concentration of the dye. The mortality of the embryos reached 55 % at the concentration of 100 µg / mL. Other effects observed in the test were edema of the yolk sac, scoliosis and malformation of the swim bladder. Predictive models of chemical toxicity, elucidation of mechanisms of toxicity, and adverse reactions that could affect the regular development of human organs have already been correlated to the effects observed in the zebrafish embryos test[3]. The Comet Assay showed statistically negative results for dye concentrations between 0.5 and 5,000 µg / mL. **Conclusion:** The positive results for the mutagenicity tests described in the literature[2], together with the positive results that we obtained in our work with the zebrafish embryo acute toxicity test, and the repeated identification of the "Disperse Violet 93" dye in textile effluents[1] allows us to classify this dye as having potential risk to human health. New studies will define the magnitude of this toxicity to humans, as well as its ecotoxicity.

References:

1. D. P. Oliveira et al. *Mutat. Re.s* 626:135, 2007.
2. G. D. A. Umbuzeiro et al. *Chemosphere* 60:55, 2005.
3. N. S. Sipes et al. *Birth Defects Res.* 93:256, 2011.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 33- *IN VITRO* GENOTOXICITY OF NITROIMIDAZOLES AS A TOOL IN THE SEARCH OF NEW TRYPANOCIDAL AGENTS

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Introduction: Chagas disease and sleeping sickness are considered important but neglected human diseases in terms of public health, whose therapeutic arsenal is restricted to undesirable high toxicity drugs, including mutagenic potential. The Institute of Pharmaceutical Technology (Farmanguinhos), located at Fiocruz, has been developing projects of new molecules with possible trypanocidal activity synthesizing nitroimidazole compounds analogs to megazol (1) using the bioisosterism, in an attempt to obtain drugs that cause fewer side effects and lack of mutagenicity. The compound 1 has potent trypanocidal activity but cannot be used clinically due to their mutagenicity. **Objective**: Evaluate if the cytotoxicity and genotoxic effect was abolished in 7 nitroimidazoles compounds (2-8) analogs of 1, employing the cell viability assay mediated by ethidium bromide (8mg / ml) and fluorescein diacetate (30µg / ml) and *in vitro* alkaline Comet assay (single cell gel electrophoresis assay) in human blood cells. **Materials and Methods**: Prior to the Comet assay, the cell viability assay in human blood cells was performed. The treatment duration with the test compounds and the solvent-control (5% DMSO) in both assays was 2 h at 37° C with concentrations in the range of 380-4000 µM for 1, and of 149-10000 µM for compounds 2-8 in 5% DMSO. In the Comet assay, methyl methane-sulfonate (160 µM) was used as a positive control. To check the cell viability, 200 cells were analyzed with a fluorescence microscope, and the extent of DNA damage in 100 cells by culture in duplicate was quantified according to the length of the tail of the comet in 4 grades (0-3). **Results and Discussion**: Compounds of 1 and 6 proved to be non-cytotoxic in human blood cells *in vitro*, whereas the other compounds (2-5, 7 and 8) showed low cytotoxicity (range 1 to 4%). It was found that there is highly significant genotoxicity of 1 in the concentrations of 1562 uM, 2500 uM and 4000 uM ($p < 0.01$) without decrease in cell viability. All the analogous compounds of 1 were genotoxic, in which 3, 4 and 7 induced significant DNA strand breaks ($p < 0.05$), and 6 and 8 caused highly significant DNA damage ($p < 0.01$), except for the compounds 2 and 5, which did not show genotoxicity when compared with the control solvent, within the range of the concentrations used. **Conclusions**: The replacement of the sulfur atom on the thiazole nucleus of 1 by nitrogen atom in the triazole nucleus present in 2 abolished the genotoxic effect. The substitution of the thiazole nucleus present in 1 by the imidazole ring present in 5, and the change of the nitro group to the 4th position abolished the genotoxic effect.

MC 34- EVALUATION OF CYTOTOXIC ACTIVITY OF RESVERATROL IN SOME CANCER CELLS

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Introduction: Cancer is a principal cause of death worldwide and according to the recent International Agency for Research on Cancer report, an estimated 14.1 million new cancer cases occurred in 2012. Breast cancer was by far the most common cancer diagnosed in women (151 countries worldwide) and the second most common cancer type was cervix cancer (30 countries in worldwide). Epidemiological studies indicate that populations consuming high levels of plant derived foods have low incidence rates of various cancers. Resveratrol (RV) is a polyphenolic compound, naturally found as an ingredient in many plant species including grapes, peanuts, mulberries. It has become an interesting and attracting subject for the research because of its role as a cancer preventive, cardioprotective and neuroprotective features. **Objective**: In this study, we aimed to evaluate the cytotoxic activity of RV by Neutral Red Uptake (NRU) Assay in different cell lines including MDA-MB 231 (Human Breast Adenocarcinoma), HeLa (Human cervical cancer) and BT-474 (Human Breast Carcinoma) cell lines. **Material and Methods**: Cells were exposed to RV (0-400 µM) for 24 hours. NRU assay was performed following the protocols described by Saquib et al. (2012). The absorbance was measured in a microplate reader at 540 nm. **Results**: Data about the protective effects of RV is contradictory. There are some studies that have showed preventive effects of RV in different cancer cell lines by using different cytotoxicity assays. But also there are reports that has not found protective effects of RV in cancer cell lines. In our study, although a concentration dependent decrease was seen in the survival of the HeLa, BT-474 and MDA-MB 231 cells exposed to RV in the concentrations studied, RV was not found to be cytotoxic to these cancer cell lines. **Conclusion**: We found that RV has less cytotoxic effects in these cancer cell lines studied. It seems that RV has no protective effects to human cervical and human breast cancer. Our results show that attention must be given in the usage of phenolic compounds in different disorders especially in cancer types. But further investigations such as using more cell lines and more cytotoxicity assays and incubations with various concentrations at many time points should be performed to confirm beneficial effects of resveratrol. Also in vivo studies are needed.

References

1- Saquib Q et al, Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells, Toxicology in vitro, 26, 351 (2012)

2- Xiang L et al, Health benefits of wine: don't expect resveratrol too much, Food Chem., 156, 258 (2014)

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 35- MUTAGENIC POTENTIAL OF WATER FROM THE BOQUEIRÃO DE PARELHAS DAM, BRAZIL DUE TO GEOGENIC HEAVY METALS AND RADIOACTIVITY

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The contamination of water bodies by heavy metals and ionizing radiation is a critical environmental issue, which can affect water quality and thus human health. This study aimed to evaluate the water quality of the Boqueirão de Parelhas Dam one of the most important water reservoirs located in the Brazilian semiarid region. A one year monitoring was performed to assess the impact of the regional geology and the anthropogenic activities on water quality in a particular scenario characterized by restricted water availability. The study was performed through the assessment of physicochemical parameters, heavy metal content and radioactivity along with a mutagenicity potential water assessment using Micronuclei Test in *Orechromis niloticus* (*in vivo*) and the Cytokinesis-Block Micronucleus Assay in human lymphocytes (*in vitro*). A deterioration of water organoleptics characteristics by the presence of high levels of sulfate and total solids was observed. High concentrations of aluminum, nickel, silver and lead along with the alpha particles content were higher than the limits suggested by the World Health Organization and Brazilian legislation for drinking water. An increase in the frequency of micronuclei and nuclear abnormalities was observed in both experimental models. The results obtained confirmed the mutagenic potential present in water samples. The results highlight that geogenic agents affect water quality becoming a human health concern to be taken into account due to the relevancy that this water resource has in the region.

MC 36- NATURAL RADIOACTIVITY AFFECTS WATER QUALITY IN REGIONS FROM THE BRAZILIAN NORTHEAST WITH RESTRICTED WATER AVAILABILITY

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The Brazilian semiarid region covers a surface of one million of Km^2, 12% of the national territory. This region is characterized for long periods of drought. This adverse scenario led to build water reservoirs (dams), aimed to counteract the hydric needs of the population. The presence of crystalline (metamorphic and granitic) rocks in the terrestrial crust can lead to enrich water sources with different radionucleides affecting water potability. The present study aimed to assess the impact of natural radioactivity on water quality of two dams located in the Rio Grande do Norte State/ Brazil. To characterize the radioactive scenario the determination of radionucleides in samples of soil, ar and water were performed. On this context concentration of radon (Rn), uranium (U), thoronium (Th) and potassium (K) along with total alpha and beta particles were determined. To assess the potential impact of radioactivity on water quality the mutagenic potential of water using Micronuclei Test in *Orechromis niloticus* and *Tradescantia pallid* were performed. A High levels of Radon in soil (range: 33750 Bq/m^3) along with U, Th and K concentrations (mean: 2.70ppm; 1.17ppm; 4.07ppm respectively) were observed. Air radon concentration varied between 4 to 311 Bq/m^3. Besides high levels of radon in water (mean 29.0 Bq/L; range 47.0 Bq/L) along with the concentration of Alpha and Beta particles was observed. A high genetic instability was observed through the three models applied. The results obtained in this work revealed a clear mutagenic potential of water caused at least in part due to the natural radioactivity present in the region.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 37- POTENTIAL EVALUATION MUTAGENIC THROUGH THE MICRONUCLEUS TEST IN RATS SUBMITTED TO EXTRACT CHRONICALLY OF *Brugmansia suaveolens*

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Introduction: The *Brugmansia suaveolens* belonging to the *Solanaceae* family, their toxicity is related to the presence of tropanic alkaloids. **Objective:** The aim of the study was to evaluate the mutagenic potential of micronucleus test in rats chronically subjected to different concentrations of *Brugmansia suaveolens* extract. **Materials and Methods:** In experimental study wistar rats (n=30) over a period of 40 days (CEUA - IPA 23/2013). The rats were divided into 5 groups: control group (CG): physiological sodium chloride 0.9%. The control of the alcoholic group (GCA): 60% ethyl alcohol; Group 50 (G50): 50mg/kg; Group 100 (G100): 100mg/kg and Group 150 (G150): 150 mg/kg of the plant extract. The animals were sacrificed and then taken to perform the micronucleus test in the trunk blood. Statistical analysis was performed by ANOVA with Tukey test, considering $p < 0.05$ statistically significant. **Results and Discussion:** Observed that the GC, GC and G50 showed a statistically significant difference when compared to the G150; CG and G150 showed statistically significant differences when compared to the G100 group. We conclude that the Brugmansia suaveolens extract has mutagenic potential in high concentrations. **Conclusions:** More studies should be done, as there is growing Brugmansia suaveolens consumption as it is an easy retrieval plant and proven toxicological effect.

UNITERMS: Tropanic alkaloids. *Brugmansia suaveolens*. Erythrocytes. Mutagenesis. Micronucleus test.

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MC 38- CARCINOGENICITY AND ANTICARCINOGENICITY EVALUATION OF THE NEOLIGNAN ANALOG 2-(4-NITROPHENOXY)-1PHENYLETHANONE

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Introduction: Neolignans are secondary metabolites found in various groups of angiospermas, which represent a class of natural compounds with a great diversity of chemical structures and pharmacological activities. These compounds are obtained by linking of two phenylpropanoid units. Several compounds which have ability to prevent genetic damage have been isolated from plants, and can be used to prevent or delay the initiation of neoplasia development. The toxicological genetic evaluation is widely used in risk assessment of new drugs in preclinical screening tests. **Aim**: In the present study we evaluated the carcinogenicity and anticarcinogenicity of the neolignan analog 2-(4-nitrophenoxy)-1-phenylethanone by the test of epithelial tumors detection in *Drosophila melanogaster*. **Materials and methods:** The neolignan 4NF was obtained by the reaction between phenacyl bromide and *p*-nitrophenol, and its formation is supported by nuclear magnetic resonance of ^{1}H and infrared. To obtain *wts* +/+ *mwh* heterozygotic larvae, virgin females *wts*/TM3, *Sb*[1] were crossed with *mwh*/*mwh* males. The larvae from this cross were treated with 3mL of neolignan analogue 4NF at concentrations of 1000 $\mu g.mL^{-1}$, 500 $\mu g.mL^{-1}$, 200 $\mu g.mL^{-1}$, 100 $\mu g.mL^{-1}$, 50 $\mu g.mL^{-1}$ and 10$\mu g.mL^{-1}$ dissolved in 2% DMSO. For the anticarcinogenicity evaluation was used MMC (0.05 mM). The larvae exposed at MMC were pretreated for 6 h. However, only adult flies, without the chromosome balancer (TM3, *Sb*[1]) were analyzed. The flies were examined using a stereoscopic magnifying glass. The tumor frequency was calculated as the number of tumors in *wts* +/+ *mwh* flies. **Results and discussion:** The test for detection of epithelial tumors in *D. melanogaster* showed that all concentrations induced an increased in the frequency of tumors ($p < 0.05$). The evaluation of anticarcinogenic potential of neolignan analog 4NF showed significant difference between treatments compared with positive control group ($p < 0.05$), in which the concentrations of 100 $mg.kg^{-1}$, 200 $mg.kg^{-1}$, 500 $mg.kg^{-1}$ and 1000$mg.kg^{-1}$ reduced significantly the number of epithelial tumors in *D. melanogaster*. Similar results were found in the studies with saffron (*Curcuma longa L.*) and soursop (*Annona muricata*) extracts, by detection of epithelial tumor in *D. melanogaster*, and these assays also showed carcinogenic and anticarcinogenic effects. Results like these are known as Janus effect, which present both carcinogenic and anticarcinogenic activities. **Conclusion:** The neolignan analog 4NF demonstrated carcinogenic action by detection of epithelial tumors test in *D. melanogaster* and showed anticarcinogenic activity against MMC detected by the epithelial tumor test in *D. melanogaster*.

keywords: Drosophila melanogaster, lignoids, secondary metabolites, Tumor, Warts.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MC 39- MUTAGENICITY INDUCED BY RADON GAS USING THE MICRONUCLEUS TEST *(Tradescantia* sp. CLONE KU-20)

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Introduction: The first observations over the existence of radon gas (Rn), initially known as "thorium emanation", were carried out between the end of 19th and beginning of 20th centuries. A result of uranium-238 (U238) radioactive decay, radon is a tasteless, odorless and colorless gas under room temperature, with a 3.825-day half life and particle α emission in its decay, and as final product of its disintegration, the stable lead-206 isotope (Pb206). Being it is the gas with the highest density known, closed and poor ventilated environments are favorable to its accumulation, with its inhalation being the highest health risk. The use of vegetal bioindicators has shown to be excellent on the monitoring of air quality and on mutagenic potential of various pollutants contained in the atmosphere. **Objective:** Within this context, the objective of this study was to evaluate the micronucleus test application potential utilizing the Tradescantia sp. clone KU-20, in order to evaluate genetic alterations induced by radon gas. **Materials and Methods:** Stems of Tradescantia sp. clone KU-20, previously immerse in Hoagland solution, were introduced in a radon detection equipment's calibration chamber (Alphaguard), containing radium salt. Afterwards, the accommodated stems were exposed to radon gas (the average radon concentration was 7.639 KBq/m3) for 24 hours. **Results:** The results demonstrated an increase on micronucleus formation (39.23 + 2.143 MCN/100 tetrads) in stems exposed in relation to the negative control (18.00 + 1.396 MCN/100 tetrads). The difference between the values indicated a significant increase on micronucleus frequency in the inflorescences subjected to radon gas. **Conclusion:** The presented results demonstrated the micronucleus test application potential using Tradescantia clone KU-20 to evaluate genetic effects induced by radon gas.

Keywords: *Tradescantia* sp., mutagenicity, micronucleus test, radon gas

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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ENVIRONMENTAL TOXICOLOGY AND ECOTOXICOLOGY

A | R | T
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EN 01- CYTOGENETIC RESPONSE OF *Tradescantia pallida* AFTER EXPOSURE TO WATER OF A IMPACTED LACUSTRINE ENVIRONMENT

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Introduction: The biomonitoring studies allow identify the combined effects of substances and evaluate their influences on organisms. Thus, they complement the monitoring based on physical and chemical analysis, since these indirectly infer the biota's effects. Plants like *Tradescantia pallida* provides genetics assays testing sensitive to environmental pollutants. So they are reliable tool used in studies of cytogenetics damage for the evaluation of aquatic environments. **Objective:** The current study aimed to evaluate Juara pond (Municipality of Serra / ES) water quality in three sampling stations, during two campaigns, through the quantification of metals and cytogenetic responses in *T. pallida* specimens exposed to water samples. **Materials and Methods:** From surface water samples, the concentrations of metals were analyzed by mass spectrometry and were performed the assay of mitosis in root tip *T. pallida.* The slides of the meristems were assembled by Feulge method and 5000 cells were evaluated for each treatment. Were calculated the mitotic index, the chromosomal aberrations index and the frequency of micronuclei that enabled the analysis of cytotoxic, genotoxic and mutagenic potential, respectively. For statistical analysis of metals was utilized Kruskal-Wallis test ($p<0.05$) and for cytogenetic data, ANOVA followed by Tukey ($p<0,05$). **Results and Discussion:** The quantification of metals demonstrated that Fe presented higher levels than established by CONAMA 357 resolution. Such levels seem to have a relationship with organic composition of the stations, since the most organically impacted station showed lower levels of Fe. In this aspect, complexation processes with organic matter might occur, reducing the concentration of dissolved metal in water. The cytogenetic tests demonstrate that two sampling stations, at least one campaign, showed significant cytotoxic, genotoxic and mutagenic potentials. C-metaphase, chromosomal adherence, lobulated nucleus and nuclear bud were significantly the most observed chromosomal aberrations in these cases, although it does not seem related to Fe levels. However, considering the occupation of watershed of Juara pond, it is suggested that the observed damage is a reflection of other pollutants, such as those present in complex mixtures of industrial and domestic potentially emitted into the pond or its tributaries. **Conclusion:** The test system *T. pallida* responded to the potential risk of Juara pond, encouraging its use coupled with usual abiotic parameters. Thus, it is emphasized the need to integrate both methods to the full assessment of water quality.

EN 02- TOXICITY EVALUATION OF CHEMICAL GROUPS DICARBOXIMIDA FUNGICIDES IN MERISTEMATIC CELLS OF *Lactuca sativa* .

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Introduction: With the growing development of technologic and agriculture, humanity tends to emit more pollutants in the environment, among which stands out the pesticides. Such substances cause environmental impacts, because leached affect groundwater and surface water. **Objective:** The study aimed to assess the genotoxic potential of the pesticides procymidone and iprodione and its mechanisms of action and the chemicals groups of dicarboximide in meristematic cells of *Lactuca sativa.* **Materials and Methods:** Seeds of *Lactuca sativa* were germinated in petri plates treated with different concentrations of two pesticides: Procymidone (31,25; 62,50; 125; 250 e 500 µg/mL) and Iprodione (46,88; 93,75; 187,5; 375; e 750 µg/mL). As positive control was used trifuralina. As negative control, distilled water. The plates were kept in a germination chamber at 24°C for 96 h. Subsequently, 20 roots per treatment were fixed in a solution of ethanol and acetic acid (3: 1). For cytogenetic analysis slides were prepared by crushing technique and stained with acetic Orcein 2%. Were prepared 10 slides per treatment and 1000 cells per slide were analyzed totaling 10000 cells per treatment. The mitotic index and the percentage of cells with chromosomal aberrations and nuclear was obtained. The averages were submitted to ANOVA and compared by Dunnet test ($p < 0.05$). **Results and Discussion:** The two pesticides, procymidone and iprodione showed a significant decrease in the mitotic index, a reduction of 10,87 and 20,64 respectively of highest concentration compared to the negative control. All concentrations of the two principles have increased the percentage of nuclear aberrations and were similar to the positive control. Considering the types of chromosomal aberrations observed, both pesticides showed the occurrence of chromosome lost and fragments, c-metaphase, bridges, c-metaphase polyploids and chromosomes sticky, the latter one was more frequently on both. **Conclusion:** The fungicide procymidone and iprodione showed cytotoxic potential, decreasing the mitotic index and genotoxic and increasing the occurrence of nuclear aberrations and chromosomal aberrations.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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EN 03- EVALUATION OF FITOTOXICITY IN A URBAN STREAM OF JABOATÃO DOS GUARARAPES CITY, PE

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Introduction: The pollution of rivers and streams, especially those located in urban areas, worsens gradually. Lack of sanitation in many communities favors the irregular dump sewage increasing the amount of organic material that can come to unbalance the ecosystem. Ecotoxicological tests, through bioassays, are widely used to monitor the toxicity of pollutants in waters impacted by effluents of various types, highlighting the phytotoxicity tests have the advantage of its low cost, variety of parameters and reproducibility. **Objective:** To evaluate the phytotoxicity of an urban stream located in the community Cristo Redentor II in Curado, Jaboatão dos Guararapes, Recife, PE in *Eruca sativa.* **Materials and Methods:** Water samples were collected at three different points of the urban stream of Cristo Redentor II: the first in more urbanized area, second in the middle region and third in the region near a patch of forest. Five Petri dishes, filled with filter paper were prepared for each site, were samples of 4 mL were added and inoculated with 10 seeds per plate and incubated for five days at constant light and 25°C temperature. The negative control was also conducted in five repetitions with mineral water. The positive control corresponded to a potassium dichromate solution at 0.1ppm. The parameters evaluated were germination, percentage change germination, inhibition rate of Germinability (IRG) and Root Growth Inhibition Rate (RGIR). The samples were also subjected to culture in nutrient medium to evaluate the microbiological quality. **Results and Discussion:** The germination was 92% P1, P2 94%, and 100% P3. Compared to the control, the percentage of germination variation was 2.22% P1, P2 4.44% and 11.11% P3. IRG P1 was -2.22%, -4.44% -11.11% P2 and P3, respectively. GIRR of P1 corresponded to 26.99%, and -12.77% and -30.61% for P2 and P3. For the last two parameters, positive values indicate growth inhibition and negative values indicate growth stimulation. The seeds germination was progressive from the point 1-3 and this progression was followed by other parameters. Points 2 and 3 showed stimulus for both IRG and for GIRR, however, despite the present point 1 stimulation on the germination, it does not follow the development of the plant that was crippled precisely the point of greater urbanization. The culture still showed the presence of *E. coli* and *Salmonella sp.* **Conclusion:** The urbanization in the vicinity of rivers and streams becomes harmful due to increased organic matter in the same as a result of sewage dump. This increase may favor the process of eutrophication and changes in the local ecosystem. Another problem is the presence of microorganisms in unbalanced amount becomes pathogenic causing risks not only to wildlife but mainly to human health.

EN 04- TOXICOLOGICAL INFLUENCE OF CADMIUM AND LEAD ON MORPHOLOGY AND GROWTH OF *Allium cepa*

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Introduction: The presence of heavy metals into streams and rivers is widely discussed. Effluents of various types such as textiles, pesticides and urban pollutants, cause increasing in the rates of metals on waters. The environmental toxicity of metallic ions must be accompanied due to physiological and biochemical damage in organisms, which can result in loss of essential molecules activity and their cumulative nature and magnification through the food web. The effects of toxic chemicals can be monitoring by the use of plants such as *Allium cepa*, which are considered an efficient test system due to its sensitivity. **Objective:** Investigate citotoxicity of lead sulphate and cadmium chloride aqueous solutions on root morphology and growth and genotoxicity in *Allium cepa* system. **Methods:** The bulbs were exposed to concentrations of metals in test tubes with 50 mL capacity for a period of 168 hours. The tests were performed in triplicate for each concentration of both metals. For the Lead Sulfate concentrations were 0.5 mM, 1.0 mM, 2.0 mM, 3.0 mM, 4.0 mM and 5.0 mM. Cadmium chloride: 0.5 mgL, 1.0 mgL, 10 mgL, 20 mgL and 50 mgL. Positive and negative controls were performed by using potassium dichromate (final concentration 1ppm) and mineral water, respectively. After the experimental period, roots from each sample were cutted off. The number and length of each root was measured. The morphological aspects of roots were evaluated by light microscopy. For genotoxicity evaluation, roots were fixed in carnoy solution for 24h and washed in destilled water after that period. The root tips were macerated in HCl 1N during 10 minutes at 600C. Samples were stained with Feulgen for 1 hour at dark and squashed in 45% acid acetic. Slides were prepared from each onion. **Results and Discussion:** *A. cepa* bulbs exposed to cadmium chloride showed no growth, indicating a highly toxic nature, causing deleterious effects even at low concentrations. Plants exposed to lead sulphate had an average root elongation and number related to metallic ion concentrations. In the presence of 1.0mM root growth was increased by 40% compared to control sample. The highest concentrations induced alterations in roots morphology and in the cellular division. The biological changes resulting from the exposure to these metals can lead to cellular death, as they can not be removed or remedied and influence the whole ecosystem. **Conclusion:** In this study, cadmium presented a high citotoxicity and genotoxic effect on *A. cepa.* Lead presented influence on the parameters evaluated related to its concentration. Microscopic study revealed alterations on root morphology and a significant reduction on mitotic index, suggesting a metal influence on the mitosis cycle, affecting the root growth and morphology.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EN 05- SCREENING OF NATIVE FISHES FOR DERIVING AQUATIC LIFE CRITERIA

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Introduction: Screening of sensitive test organisms plays a fundamental and critical role on deriving aquatic life criteria. Test organisms should be selected according to the characteristics of target regions and biota differences. However, the screening of sensitive test organisms were rarely investigated, though different countries require different number of test species used for water quality criteria establishment. For instance, The WQC derivation in USEPA requires at least eight animal families of three phyla. Currently, in China, the test organisms selected are mainly referred to those of other countries, which may result in the overprotection or underprotection for the Chinese native species due to the biota difference. Therefore, it is urgent to conduct the systematic research on the sensitive test organisms screening in order to establish more scientific and reasonable aquatic life criteria for China. **Objective:** Fish is one of the important protection objects of water quality criteria. Our study aims to develop a method to screen native sensitive test organisms and take fish as an example to explain how to find native species sensitive to corresponding pollutants. **Materials and Methods:** Seventeen native representative fishes were selected mainly based on their geographical distribution, quantity of the toxicity data. According to the data collecting principle of aquatic life criteria, the toxicity data of these fishes were collected and screened from ECOTOX, CNKI and published literatures. The list of pollutants most toxic to fish was obtained through species sensitivity analysis. They included pesticides, chlorphenols, heavy metals and ammonia. According to the cumulative probability of each fish from fish sensitivity distribution curves of the most toxic pollutants, species sensitivity was classified. **Results and Discussion:** Ten fishes among the seventeen fishes were selected to be the test organisms as the native fishes for deriving water quality criteria. The fished included Cyprinidae species: *Cyprinus carpio* for fenvalerate and thiram, *Ctenopharyngodon idellus* for triazophos, *Hypophthalmichthys molitrix* for fenpropathrin, *Aristichthys nobilis* for cadmium and dichlorvos, *Carassius auratus* for inorganic mercury, *Pseudorasbora parva* for organophosphorus pesticides; and non-Cyprinidae species: *Misgurnus anguillicaudatus* for dichlorvos, *Pelteobagrus fulvidraco* for dichlorvos and omethoate, *Monopterus albus* for cypermethrin, *Mugilce phallus* for endosulfan and fenvalerate, *Siniperca chuatsi* for non-ionic ammonia. **Conclusions:** These species above can be considered as native sensitive fishes for development of aquatic life criteria for corresponding pollutants.

EN 06- RESEARCH PROGRESS OF WATER QUALITY CRITERIA IN CHINA

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Introduction: Water quality criteria (WQC) are fundamental for developing enforceable water quality standards (WQSs) and play an important role in management of water quality and aquatic ecosystems around the world, especially in China where the situation of water pollution is getting grimmer and grimmer. China's current water quality standards are formed on the basis of foreign water quality criteria. From 2008, Chinese government decided to set up its own water quality criteria system to support its environmental management. After several years' research, some significant progresses have been achieved for development of China's WQC. The presentation introduces main research progress of WQC in China. **Current Worldwide Research Status of WQC:** The USA was the first country to start the formal research on WQC. The US Environmental Protection Agency has released a series of guidelines for deriving WQC. EU and other developed countries have also issued related guidelines. The mathematical derivation method incorporating the SSD has been most widely used to derive criteria or standards values. To protect aquatic life and human health, most countries set WQC or WQS refer to the experience of the US or EU. **Research Progresses of WQC in China:** At present, the methodological framework for WQC has been preliminarily constructed. Some key technologies for deriving WQC have been researched to promote the establishment of technical systems for WQC in China, such as the "Minimum Toxicity Data Requirement" of "6 families in 3 phyla" and "10 species in 4 phyla", the "biological effect ratio (BER)" technique, and the methodology for deriving WQC for combined effects of multiple pollutants. WQC threshold values for representative pollutants and nutrients in water environment have been derived to provide solid basis for the amendment of "Environmental Quality Standards for Surface Water" in China. According to SSD approach and the corresponding method used in ecological risk assessment, the emergency WQS of 6 representative heavy metals were derived to be as technical references for accidental pollution events by heavy metals in aquatic environments in China. All these achievements and experience lay down the foundation for further improvement of WQC and WQS in China. **Prospective:** More researches are still needed to further improve and perfect the WQC system in China, such as, the construction of the technical systems for WQS and the establishment of the technological systems for transforming WQC into WQS, the construction of the database for WQS in China, improvement of WQC/WQS related environmental policies, regulations and laws.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 07- GLYPHOSATE-BASED HERBICIDE ALTERS BEHAVIORAL PARAMETERS AND CATALASE ACTIVITY IN ADULT ZEBRAFISH (*Danio rerio*)

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The global and domestic demand for pesticides has increased significantly in recent years. However, they generally persist in the agricultural products and in the environment, posing health hazards to humans and animals. It has been demonstrated that glyphosate-Roundup® is the major herbicide used worldwide and its toxic events might be due to the synergistic effects between glyphosate and other formulation products. **Objective:** The purpose was investigate the effects of acute glyphosate-Roundup® exposure on behavioral parameters and catalase activity in zebrafish. **Methodology:** Adult zebrafish (*Danio rerio*) of both sexes were exposed to glyphosate-Roundup® (GR; 0.5 or 5.0 mg/L of active ingredient) during 96 h. After that, they were transferred to the light/dark preference test for 6 minutes and videotaped. The videos were analyzed with the program AnyMaze®. Following the behavioral tests the animals were euthanized with a solution of 250 mg/L of tricaine. Brain and liver were collected for analysis of the catalase (CAT) activity by using the hydrogen peroxide (H_2O_2) as substrate. All procedures were approved by the Ethics Committee for Animal Use, Unochapecó (Protocol 005/14). **Results and Discussion**: The exposure to both concentrations of GR for 96 h significantly reduced the number of crossings (p: 0.0435; n: 18) and number of entries in the lit area (p: 0.0009; n = 18) compared to control group (without herbicide). In opposition,5.0 mg/L of GR significantly increased the latency for the first crossing (p: 0.0053; n =8) although there was no change the time spent in the lit or dark areas. These results indicate that acute exposure to the herbicide did not change parameters related to anxiety since no changes were observed in the time spent in the dark side but the reduction in the number of crossings is an indicative of motor abnormality. In addition, it was observed a significant reduction on CAT activity in brain (p: 0.0083; n: 4) after exposure to 0.5 and 5.0 mg/L GR while there was an increase in CAT activity in the liver from animals exposure to 5.0 mg/L GR (p:0.0307; n:4) suggesting an imbalance in antioxidant defenses. **Conclusion:** Zebrafish exposed to GR did not show anxiolytic-like behavior in the light / dark test, however occureda reduction in motor activity. Moreover, the changes observed in CAT activity in brain and liver suggest that the herbicide may lead to oxidative stress but more studies are necessary to better understand this effect.

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EN 08- ARSENIC METHYLATION CORRELATES WITH LIVER DYSFUNCTION IN MICE MODEL

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Introduction: Chronic exposure to arsenic (As) through drinking water is associated with many health events. The different metabolic products of inorganic arsenic will lead different hepatoxicity. Arsenic, a well known exogenous stressor and hepatoxicant[4], could create a state of oxidative stress in liver. While there are no reports on the relationship between the arsenic metabolic methylation process and liver function and oxidative stress. **Objective:** This study was designed to explore the relationship between arsenic methylation and liver oxidative stress induced by arsenic trioxide (ATO). **Materials and Methods:** 40 healthy KM mice were randomly divided into control group (0.9% saline), As2O3 (1.0mg/Kg/day, 2.0mg/Kg/day, 4.0mg/Kg/day) groups with gastric perfusion for 5 weeks. With high efficiency liquid chromatography and HPLC-HGAFS. The products of arsenic trioxide methylating including iAs^{3+}, iAs^{5+}, MMA, DMA in the liver were determined. The indexes of arsenic methylation including PMI, SMI were calculated. The level of hepatic function and activity of MDA, GSH, SOD, TAOC were detected with kits. **Results and Discussion:** We found that the remain of arsenic metabolic products in liver significantly increased with the increasing doses of arsenic trioxide and the liver function and oxidative stress deteriorated. The negative correlation were found between MMA%, PMI and GSH, SOD, TAOC, while DMA%, SMI positively correlated with the levels of ALT, AST. PMI and SMI negatively correlated with TAOC, GSH, SOD, ALT, AST, positively linked with the level of MDA. Our study found that the concentration of specific arsenic in mice liver increased obviously with ATO treatment, and DMA occupied most proportion. **Conclusions:** The present study demonstrates that the hepatotoxicity induced by the arsenic accounts for deteriorating oxidative injury activated by arsenic methylation metabolism, providing additional evidence to suggest a mechanism of arsenic poisoning. Therefore, reducing the process of arsenic methylation may be potentially beneficial in treating and more importantly, preventing arseniasis.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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EN 09- POLYAMINE PUTRESCINE DECREASES STEROIDOGENIC PROTEIN LEVELS

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Introduction: The putrescine is a polyamine that modulates the processes of cell growth and differentiation, beyond regulating transcription and replication of genetic material. Currently has drawn attention the contamination of groundwater caused by cemeteries. This contamination occurs mainly by percolation of necrochurume, a liquid released by the putrefying corpse, consisting of a variety chemicals, among them putrescine. Literature data regarding the environmental toxicity and potential reproductive toxicity of this substance is scarce. However, we observed a reduction in serum testosterone levels of animals after chronic exposure to this contaminant. **Objective:** To quantify by Western blotting the levels of testicular StAR, CYP11A1 and 17 β-HSD proteins involved in the pathway of steroidogenesis. **Materials and Methods:** We used 24 Wistar adult male rats orally exposed to different concentrations of putrescine (Co- water; T1- 46.3 mg/kg, T2- 138.9 mg/kg and T3- 231.5 mg/kg) by chronic period of 56 consecutive days. After euthanasia, the testes were homogenized for 30 sec and protein samples were treated with Laemmli buffer and used to run SDS-PAGE (12%). The proteins were electrotransferred to a PVDF membrane and incubated for four hours with anti StAR-specific antibodies, anti-CYP11A1, anti-17 β-HSD previously diluted (1:100) in blocking solution. After washing the membrane was incubated for two hours with anti-rabbit IgG secondary antibody diluted 1: 5000. Then it was used chemiluminescence kit and disclosed in fotodocumentador. The intensity of the bands was evaluated by densitometry. **Results and Discussion:** Effect dose response was revealed for all three studied proteins. Significant reduction was observed on the StAR levels in T2 and T3 groups, suggesting a decrease on the cholesterol molecules that penetrate in mitochondria, a process mediated by StAR protein. The CYP11A1 levels reduced significantly in the three exposed groups, suggesting that conversion of cholesterol to pregnenolone is compromised. Additionally the levels of 17 β-HSD also reduced significantly in all treatment groups, when compared to control group. This latter protein converts androstenedione to testosterone, thereby jeopardizing the production of androgen. **Conclusion:** Our results showed putrescine induced decrease on steroidogenic pathway essential proteins, leading to consequent reduction on serum testosterone level.

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EN 10- SPENT POT LINER (SPL) AND ITS MAIN COMPONENTS: GENOTOXICITY ON HUMAN LEUKOCYTES

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Introduction: The Spent Pot Liner (SPL) is a solid residue from aluminum production that has as its major components fluoride (Fl), cyanide (Cn), and aluminum (Al). **Objective:** The genotoxic potential of SPL and its main components were evaluated in this study, by the comet assay, using human blood cells. **Material and Methods:** Leukocytes from peripheral blood were obtained from healthy volunteers and incubated with SPL, fluoride, cyanide and aluminum, each tested separately. The leukocytes were then mixed with low melting point agarose, coated into microscopy slides and submitted to osmotic lysis in order to obtain the nucleoids. The slides with the genetic material of individual cells remained in rest in alkaline solution for 25 minutes to expose the alkali-labile sites with subsequent electrophoresis that allows the migration of DNA molecules. DNA fragmentation on the nucleoids was evaluated in epifluorescence microscope using visual scores after the slides were stained with propidium iodide. The comets were labeled from 0 to 4 according to their tail length and head diameter (class 0: damages from 0 to 5%; class 1: 5 to 20%; class 2: 20 to 40%; class 3: 40 to 85%; and class 4: over 85%) and the data were presented as damaged nucleoids frequency and Arbitrary Units (AU), for each treatment. **Results and discussion:** The evaluated dose matches the IC_{50} of SPL previously described (23.6 g/L), and the corresponding quantities of their fractional components Fl (0.0046 g/L), Cn (0.394 g/L), and Al (0.0031 g/L). All treatments induced DNA fragmentation. Fluoride presented an AU value near the one obtained to SPL and the positive control - doxorubicin (258, 270, and 259.7, respectively), and 15 times higher than the negative control ($CaCl_2$ 0,01M), standing out as the main genotoxic component. The aluminum presented intermediate values (AU= 165.9), and cyanide induced the lower values (AU=110.5), although this value is 6 times higher than the negative control. **Conclusion:** Both SPL and its major components proved to be genotoxic on human blood cells. Therefore, their production, storage, and disposal should be regulated and supervised with more caution.

Key Words – Spent Pot Liner, genotoxicity, Comet

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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EN 11- OXIDATIVE STRESS MOLECULAR MARKERS EVALUATION AND EPIDEMIOLOGIC ASSESSMENT OF SUBJECTS ENVIRONMENTALLY EXPOSED TO MERCURY IN THE EASTERN AMAZON

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Introduction: In the present study we report mercury (Hg) levels, evaluate polymorphisms related to the oxidative stress genes and perform an epidemiologic assessment in a population sample from Santarém municipality, located in the lower Amazonas river region. The subjects are environmentally exposed to Hg contamination by the intake of fish. **Objective:** Evaluation of the total Hg levels, in sample populations from urban and riverside (Santa Maria do Tapará) communities, in association to factors such as age, sex, and the frequency of fish intake, as well as the occurrence of deletions in oxidative stress-related genes (*GSTM1* e *GSTT1*). **Materials and Methods:** Volunteer subjects were recruited (n = 98; 30 male and 68 female), 18 – 81 years old, living in the urban area (64.3%) and at a riverside community (35.7%). The study was approved by the Ethics Committee of Pará State University and all the participants gave written consent. The study subjects filled out a health/lifestyle questionnaire. Blood samples were collected and total Hg blood concentration was determined in the DMA-80 Direct Mercury Analyzer (Milestone). *GSTM1* and *GSTT1* deletions were genotyped using multiplex PCR. Two subgroups were determined by the frequency of fish intake (high and low frequency) and three possible relationships were investigated: (a) Hg levels and frequency of fish intake; (b) Hg levels and genotypes; and (c) Hg level and age only for subjects with higher frequencies of fish intake. Respectively, for each assessment: (a) since the variances within the subgroups were not approximately equal, the ANOVA could not be used, then a Kruskal-Wallis test was performed; (b) the ANOVA was performed; and (c) an analysis of correlation was performed. **Results and Discussion:** (a) In relation to the fish intake, a significant statistical difference between the Hg level was observed ($c^2 = 30.167$; $p = 0.0001$); (b) in relation to *GSTM1* and *GSTT1* deletions, no statistical difference between Hg level was observed (respectively: $F = 2.00$, $p = 0.1624$ and $F = 1.14$, $p = 0,2896$) within the high fish intake frequency subgroup; (c) for the 66 individuals within the high frequency subgroup, a positive correlation between age and Hg level was calculated ($r = 0.39$; $p = 0.0014$), and the correlation coefficients were also positive for stratified groups of 46 females ($r = 0.36$; $p = 0.0141$) and 19 males ($r = 0.54$; $p = 0.0180$). **Conclusion:** Our results demonstrate that individuals at the eastern Amazon are environmentally exposed to Hg due to high fish intake frequency, reaching high levels of Hg in blood (above 10µg/L). Within the high level frequency group, the *GSTM1* and *GSTT1* deletions were not related to the Hg levels however a positive correlation between age and Hg levels was found.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EN 12- EVIDENCE ON THE GENETIC AND EPIGENETIC EFFECTS OF TRANSPLACENTAL EXPOSURE TO TOBACCO SMOKE

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Introduction: It is for long known that although the risks of developing chronic diseases are attributed to both genetic and environmental factors. Interactions between genes and environment may represent genetic sensitivity to environmental stressors, or, alternatively, environmental exacerbation of genetic effects. Early, the evaluation of interaction between xenobiotics and DNA has shown that chemicals can damage cells through different mechanisms being therefore designated as mutagenic, genotoxic or cytotoxic. These damage if not repaired may lead to the development of different diseases, namely, cancer. Environmental tobacco smoke (ETS) exposure is still one of most important environmental exposures and may be particularly important if one considers in utero exposure. Thus, studies based on cancer-related biomarkers of exposure and effects in umbilical cord blood have been initiated to investigate the effects of in utero exposures related to maternal exposures. **Objective:** The present study aims to review available data on DNA damage and changes in patterns of DNA methylation in newborns resulting from in utero exposure to tobacco. **Materials and Methods:** The identification of studies to be included in this review was carried out through literature search using the PubMed database. Eligible studies were those conducted in humans, written in English, reporting DNA damage or DNA methylation in newborns and/or placental tissue and with well-characterized *in utero* exposure to tobacco. **Results and Discussion:** Results showed several knowledge gaps relating, for example, to the impact of genotype, nutritional and life- style factors, methodological concerns and prospective association of DNA damage and DNA methylation with disease risk, to mention a few. On this basis, further investigations are required to provide scientific support for the possible implementation of coherent and effective health promotion and disease prevention in early stages of life. **Conclusions:** Data here obtained identifies knowledge gaps in research related to risk of exposure and maternal consumption of tobacco for the health of the newborn. Understanding the harmful effects of tobacco on the health of a developing foetus is a compelling reason to promote cessation of tobacco use during pregnancy and optimize the current and future health not only of women but also their children.

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EN 13- CADMIUM EFFECTS ON ZEBRAFISH REPRODUCTION: COMPARISON BETWEEN BRAZILIAN AND NORTH AMERICAN WATER LEGISLATION

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Introduction: the metals potentially toxic are source of environmental pollution and despite being object of scientific study a few decades, this substances are receiving in present some attention how possible endocrine disruptors (ED). In this context, the cadmium (Cd), have been attention in literature and present more preoccupation because is used alot in some anthropogenic activities, besides yours natural sources. **Objective**: evaluate the chronic effect of Cd in *Danio rerio* (zebrafish) reproduction in two different concentrations allowed by vigente legislations. **Materials and methods:** male and female of zebrafish (separately) were exposed to two concentrations of Cd, 1µg/L (BR)[1] and 0,25µg/L (USA)[2] and control (just cultive water) in 14 replicates, by 21 days in semi -static system. In the and of this período 3 female and 6 male were chosen randomly to realization of reproduction experiment (two male and one female by aquarium), and in average 12 organims to bioconcentration test. The parameter analized was, number of eggs per female, larvae viability until sisxth day, hatching rate eggs and relationship between viable born eggs and viable larvae until sixth day. Furtermore, was calculated the bioconcentration fator (BCF). **Results and discussion:** The results obtained and grounded in statistical analysis (Kruskal-Wallis) not show significant results ($p>0,05$) about number off eggs and larvae viability until sixth day. Considering hatiching hate eggs, obteined distinctis percentagens between control (93,91%) and tested concetrations (USA 71,82% e BR 99,65), the relationship of viable eggs and larvae (Test-G) presented significant difference ($p<0,05$). About BCF, obtained this values: 103,7 (BR) and 104,2 (USA), this fator relate how much was incorporated biologically by the organism and may be related to such toxicity at the and of the analysis we highlight the following: 1) the BR concentration apresented hatiching rate greather than control, 2) considering the tested concentrations, is observed a hormesis case, once the lowest concentration of contaminant (USA) is more toxic to referide reproductive parameter and 3) the obtaneid values to BCF of Cd indicated that there was a higher biological incorporation of said metal at the lowest concentration (USA), this being an indication of why the lower concentration is more damaging to the species in question. **Conclusions:** the dates of presente research indicate that Cd allowed for protection of aquatic life in international law presents reproductive injuries, therefore has potential to act as ED.The toxicological behavior of the metal refers to hormesis behavior and should be tested for other species in order to evaluate a safe concentration of aquatic biota, considering the reproductive aspects.

References:

1. CONAMA – Conselho Nacional do Meio Ambiente. **Resolução CONAMA nº 357 de 17 de março de 2005.** Ministério do Meio Ambiente. 2005.
2. USEPA - United States Environmental Protection Agency. **National Recommended Water Quality Criteria**. 2000.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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EN 14- HORMONE DEGRADATION IN WATER AND ITS IMPORTANCE TO THE EXPERIMENTAL DESIGN OF ECOTOXICOLOGICAL ASSAYS

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Introduction: The presence of endocrine disruptors (EDs) in the water has been encouraging scientific studies, especially related to compounds commonly found in the environment, such as female sex hormones (FSHs), which stand out as EDs group most investigated in environmental toxicology studies. Nevertheless, in this case, the study of chemical behavior of pollutants that should be assist in the methodological adjustments to ensure understanding of pollutant-response relationship and realize the action of by-products in ecotoxicological assays, is not being investigated. **Objectives:** This study aimed to investigate the influence of laboratory conditions generally used in ecotoxicological assay with FSHs. **Materials and methods:** Two glass aquaria replicated 2L, a first containing two fish of *Danio rerio* species and the second not, have been prepared under each experimental condition tested (control, E2 and EE2) simulating the experimental design of an ecotoxicological assay with complete exchange of culture medium every 24 or 48 hours, photoperiod and physicochemical parameters controlled and using an environmentally relevant concentration (30 $ng.L^{-1}$, nominal concentration) of hormones 17ß-estradiol (E2) and 17α-ethinylestradiol (EE2) through the time. Duplicate aliquots (250 mL) were collected from each aquarium at 5 minutes (initial time), 24 and 48 hours for analysis and quantification by gas chromatography (GC-MS). **Results and discussion:** Data analysis revealed the increasing presence of estrone (E1) produced as a byproduct of E2 degradation in water from 24 hours of exposure in the test. Were also revealed lower levels of hormones available with the presence of test organisms that probably contributed to metabolism of them. Although many contaminants are susceptible to abiotic degradation processes, such processes are often slow; faster degradation must occur by microorganisms activity. They certainly were present in higher amounts on the replicates with fish consequently of their excrement elimination in water. Results showed the impairment of maximum required concentration in 24 hours of starting the assay and the presence of degradation by-products that may influence the toxicity. **Conclusions:** These evidence suggests that preliminary studies should be conducted with the intention to better tailor the interest contaminant chemical behavior within the experimental design planned so that biological effects are not assigned exclusively to the initial contaminant and concentration, but the real pollutants present in the assay.

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EN 15- BIODIVERSITY AND ADAPTIVE EVOLUTION OF CYTOCHROMES P450 IN LORICARIIDAE FISH

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Introduction: Cytochromes P450 (CYP) genes code for enzymes involved both in the metabolism of endogenous compounds and in the biotransformation of xenobiotics. Loricariidae is a diverse family of Neotropical fish, which at least two genera (*Hypostomus* and *Pterygoplichthys*) have a modified CYP1A with reduced affinity to ethoxyresorufin, its classical substrate. **Objective**: This study aim to describe the diversity of CYP genes, its adaptive changes in the Loricariidae fish, and their regulation by xenobiotics. **Material and Methods**: Thirty-one species of Loricariidae fish and four of Callichthyidae as sampled in Rio de Janeiro and Amazon States in Brazil. The liver was excised and preserved in RNA Later until total RNA extraction, which was used for cDNA libraries preparation for Illumina sequencing. Quality of RNA extractions and libraries were accessed by Bioanalyzer. Libraries were barcoded and sequenced on eight lanes of a HiSeq2500. Transcriptome was assembled with Trinity. CYP transcripts were retrieved using BLAST with a local database of CYP genes from *Danio rerio* and other more related fish species. The sequences were edited with Seaview, aligned with Muscle and phylogenetic trees built with maximum likelihood. Adaptive evolution of each amino acid was evaluated using the online server at datamonkey.org and/or PAML. **Results and Discussion**: CYP51 of Human (gi: 168693652) and *D. rerio* (gi: 99028935) were used to root our phylogenetic analysis, that resulted in eight well supported P450 clans. The CYP2 was the most abundant P450 family in all the fish species. For example, in *Pterygoplichthys anisitsi* the complete CDS for 25 CYP2 genes were obtained. Among those CYP2 genes, expansions were identified in two subfamilies CYP2Y, with 12 distinct complete CDS, and CYP2AA, with eight distinct complete CDS. Expansion of CYP2AA was also observed in other Loricariidae species. However, the loricariids' CYP2AA appear to have evolved independently from their homologs in zebrafish (*Danio rerio*). All the species have a single CYP1A isoform, in which 19 amino acids were identified to be evolving under positive Darwinian selection. The responses of P450 genes to xenobiotics are going to be evaluated in selected species.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EN 16- IMPACTS ON HUMAN AND ENVIRONMENTAL HEALTH FROM SPENT COFFEE GROUNDS DISCARDED IN THE ENVIRONMENT

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Introduction: Coffee is one of the most widely consumed beverages around the world. According to the International Coffee Organization more than 8 million tons of this compound was consumed worldwide in 2014. So far, many studies have shown the properties of the coffee beverages, but little is known about the impacts on human and environmental health from its discard in the environment. During the processing of coffee powder with hot water around 75% of spent coffee grounds (SCG) are obtained. This residue does not have commercial value and is usually discarded reaching the environment by disposal in landfill or discharge in water bodies through sewage. **Objective**: Investigate the mutagenic, genotoxic, cytotoxic and ecotoxic effects of leached and solubilized extracts from SCG, which simulates the discard of SCG in landfill and through sewage, respectively. **Material and Methods**: Leached and solubilized extracts were obtained according to Brazilian Association of Technical Norms 10005 and 10006 respectively. The mutagenic effects were evaluated by *Salmonella*/ microsome assay using TA98 and TA100 strains according to ISO16240 in the presence and absence of exogenous metabolization activation (S9). To evaluate the genotoxic and cytotoxic effects Micronucleus (MN) and WST-1 assays were carried out, respectively, using HepG2 cell line. *Daphnia similis* was used to determine the acute ecotoxic effects. **Results and Discussion**: Leached extract from SCG induced mutagenicity in TA98 strain with and without S9 at higher dose (100% of extract) and no mutagenic response was found in TA100 strain. Solubilized extract induced mutagenicity in TA98 strain with and without S9 at 100% and from 12.5 to 100% of extract respectively. In TA100 strain solubilized extract induced mutagenicity only in the absence of S9 at 50% and 100% of extract. Micronucleus induction was observed after 3 and 24 h of exposure to both extracts and, as well as *Salmonella* assay, the solubilized one induced greater DNA damage. So, if this compound reaches a water body used for public water supply, the population may be exposed to the mutagenic effects of this compound, as well as biota. No cytotoxic effects were observed in HepG2 cells by WST-1 assay. Considering ecotoxic effects, EC50 after 48 hours for leached and solubilized extracts were 1.5%, and 11.26% respectively. This shows the hazard effects of the SCG to aquatic biota, mainly the leached extract. **Conclusion**: SCG discarded in the environment may pose a risk to human and environmental health, since this compound can damage DNA and present toxicity to aquatic organisms. Moreover, the solubilized extract is more mutagenic and genotoxic than leached; however this showed greater ecotoxicity than solubilized.

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EN 17- EPOXIDE HYDROLASES OF SUKERMOUTH ARMOURED CATFISHES (LORICARIIDAE)

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Introduction: The neotropical catfish family Loricariidae (Siluriformes) is the fifth most species among all vertebrates. Some Loricariidae fish are known to be highly resistant to organic toxins and to have cytochromes P450 with altered substrate specificities. Loricariidae epoxide hydrolases (EH) has not been investigated.EH is an ancient gene family, present in all domains of life. The encoded enzymes catalyze the hydration of epoxides to diols. EH genes are classified into five subfamilies, two of those havegreater toxicological relevance: the microsomal epoxide hydrolase (EPHX1) and the soluble epoxide hydrolase (EPHX2). EPHX1 plays a pivotal role in the detoxification of epoxides. EPHX2 shows an additional role in epoxides detoxification. **Objective**: This work aim to investigate the biodiversity of epoxide hydrolase transcriptsin Loricariidae fish family, and to evaluate their regulation by xenobiotics. **Material and Methods**: Thirty-one species of Loricariidae fish and four of Callichthyidae was sampled in Rio de Janeiro and AmazonStates in Brazil. The liver was excised and preserved in RNA Later until total RNA extraction, which was used for cDNA libraries preparation for Illumina sequencing. Quality of RNA extractions and libraries were accessed by Bioanalyzer. Libraries were barcoded and sequenced on eight lanes of a HiSeq2500. The liver transcriptome was assembled with Trinity. EH transcripts were retrieved using BLAST with a local database of EH genes from *Danio rerio* and other closer related fish species. The sequences were edited with Seaview, aligned with Muscle and phylogenetic trees built using PhyML. **Results and Discussion**: Transcriptomes of 16 fish species have been analyzed.In total,76 epoxide hydrolase sequences were found;27 of EPHX2, 48ofEPHX1 and 1 of EPHX4. Of those sequences, 35 transcripts (22 EPHX1, 12 EPHX2, 1 EPHX4) coded for more than 75% of the complete coding sequence (CDS) of their zebrafish homolog. Currently, phylogenetic relations among these sequences are being evaluated, as well as evidences of episodic diversifying selection.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EN 18- PHYSICAL- CHEMICAL AND GENOTOXIC ANALYSIS OF AN AQUATIC ENVIRONMENT UNDER HARMFUL HUMAN ACTIVITIES

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Introduction: Pollution of aquatic environments is a worldwide problem. Regions with unfavorable climatic conditions are more susceptible to harmful impacts related to human activities, increasing bioaccumulation of toxins that induce genetic damage in organisms that are in direct or indirect contact with them. The Guaribas river is one of the major rivers in the State of Piauí / Brazil, and is a source of water supply, crop irrigation and pisciculture in the Picos microregion, which includes more than 30 cities. This aquatic environment, in recent years, has suffered from intense human activities enhanced by severe climatic conditions such as low precipitation and high temperatures. **Objective:** The aim of the study was to evaluate genotoxic effects in the fish *Oreochromis niloticus*, and perform physical-chemical analysis of water samples from the Guaribas river in the city of Picos-PI / Brazil and its surroundings. **Materials and Methods**: We carried out physical-chemical analysis of surface water samples, and performed the genotoxicity assay, Comet assay, using the fish *Oreochromis niloticus* as a bioindicator model in ecotoxicological studies. The scales used were: Temporal (dry / 2013; rainy / 2014; dry / 2014 and rainy / 2015) and spatial (points upstream, within and downstream of the city). **Results and discussion:** The water quality showed that points within and downstream of the city, regardless the seasons of the year, have presented pollution levels above the acceptable according the federal legislation (ANVISA), and are statistically higher ($p < 0.05$) when compared to points upstream of the city, which presented low levels of contaminants and genetic damage in the bioindicators. The analysis during different seasons showed that pollution is present throughout the year, being more intense during the dry season. For genetic analysis, the points within and downstream of the city presented a significant increase in fragmented DNA ($p < 0.001$) when compared to an unpolluted reference point, confirming the direct relationship between pollution and genetic damage. Industrial and domestic wastes discharged in the aquatic system are the main source of heavy metals and hydrocarbons that were genotoxic to the fish red blood cells in this study. **Conclusion:** Guaribas river, throughout the year, presents itself as a polluted aquatic environment within and downstream of the city of Picos-PI, with chemicals discharges as the cause of genetic damage in fishes exposed to its waters. Thus, we strongly recommend control measures to rescue the polluted habitats, since the river is used for public water supply, irrigation, among other activities.

EN 19- EVALUATION OF ANTIOXIDANT ACTIVITY AND CYTOPROTECTION OF EXTRACTS OF *EUGENIA UNIFLORA* L. AND *PSIDIUM SOBRALEANUM*.

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Introduction: The antioxidant activity has been an important issue considering its importance on human health. Recent studies point to the use of plants in the form of juices or teas as sources of natural antioxidants that present low risk and can be used in the treatment of various diseases[(1-3)]. **Objective:** To evaluate the potential antioxidants and cytoprotective, *in vitro,* of extracts of *Eugenia uniflora* and *Psidium sobraleanum*, in addition to quantify phenols and flavonoids present in the extracts. **Materials and methods:** The antioxidant tests were performed by methods of TBARS and DPPH, the cytoprotection test was conducted according to the methodology off Shadomy, Espinelingroff and Cartwright, 1985(modified) [(4)] and the trials of phenols and flavonoids in accordance with the methodology of Woisky and Salatino1998 [(5)], and Singletonet al.1999[(6)] . **Results and Discussion:** There is a better antioxidant activity to extract *Eugenia uniflora*, for the TBARS assay with egg phospholipid , extracts reduced basal levels of lipid peroxidation process CE_{50} 185.47 mg / mL, and when Fe^{2+} induced by the extract of *Psidium sobraleanum* it was more efficient with CE_{50} of 80.45 mg / ml. Both extracts showed an cytoprotective effect on the bacterial cell when combined with heavy metal. The *E.uniflora* extract had the highest percentage of flavonoids when compared to standard, while *P. sobraleanum* extract had the highest percentage of phenols when compared to standard. **Conclusion:** Through these tests can verify that the extracts from the leaves of the species, *Eugenia uniflora* and *Psidium sobraleanum*, feature an antioxidant activity directly related to phenolic substances produced from your secondary metabolism.

References:

1. Silva, C.G et al., Pharmacol Res Commun, 52, 229 (2005).
2. Sabir, S. M. & Rocha, J. B. T, Food Chem, 111, 845 (2008).
3. Sanchez-Moreno, J.A. Larrauri, F. Saura-Calixto. J Sci Food Agric, 76, 270 (1998).
4. Shadomy, S.; Espinelingroff, A.; Cartwright, R, Manual of Clinical Microbiology, 4, 991(1985).
5. Woisky, R. & Salatino, A. J. Apic. Res. 37, 99 (1998).
6. Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Academic Press: Orlando, 299, 152 (1999).

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A | R | T
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EN 20- OXIDATIVE STRESS INDUCED BY MERCURY EXPOSURE IN CHILDREN AND YOUTH RIVERINE POPULATION FROM MADEIRA RIVER (RO/BRAZIL)

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Introduction: The Amazon region is building important economic activities such as Hydroelectric plants. In the Madeira basin Rondônia (RO) we have two Hydroelectric plants (Santo Antônio and Jirau) under construction, in the municipality of Porto Velho. This economic activity has positive impacts such as, increase of job, income and energy, but there are also social and environmental impacts that can change the quality of life of the local people and the morbidity and mortality profiles. Mercury (Hg) is present in Amazonia soils and can be mobilized from this compartiment through the flooding of large areas of dams. Methylmercury (MeHg) is the most toxic form to humans and bioaccumulates in the food chain causing health problems. Mainly for the central nervous system (CNS). **Objectives**: To assess mercury exposure via fish ingestion, in children and youth riverine population on the Madeira river, and its association with biomarkers of oxidative stress, comparing with urban populations of the city of Porto Velho. **Materials and Methods**: A cross-sectional study was carried out. Children and youth (n=198), between 5 and 17 years old were selected in 3 communities (Belmont and Cunia riverine communities and an urban community). Blood and hair were collected for determination of mercury in the hair (Hg-H) and blood (Hg-B). Serum samples were obtained to determine the activity of the enzyme glutathione S-transferase (GST), and concentration of thiol groups and malondialdehyde (MDA). The analyzes of Hg were carried out by CV AAS (Hg-H) and ICP-MS (Hg-B) and oxidative stress analyzes were by Spectrophotometry UV-Vis. The confidence level was 95%. **Results and Discussion**: The population with higher Hg levels and oxidative stress was the riverine community of Cuniã, with the highest frequency of fish consumption. The biomarkers Hg-B, Hg-H, MDA and GST were significantly different (Tests T and Mann-Whitney, p -value < 0.001) between the three communities studied, being higher in Cuniã. The Thiol biomarker did not differ between communities. The levels of Hg-B and Hg-H in Cuniã were above the WHO reference values for non-environmentally exposed populations (8 µg/L and 2 µg/g, respectively). Riverine communities with diet rich in fish have the highest risk of Hg exposure. **Conclusion**: This study shows the importance of monitoring programs of Hg exposure for the health. Biomarkers of oxidative stress can bring an important overview of early metabolic abnormalities related to environmental exposure, but it is necessary to have a specific biomarkers in order to increase the reliability of results.

EN 21- ASSESSMENT OF THE IMPACT OF TEXTILE EFFLUENT ON WATER QUALITY OF RECEIVING RIVERS IN URBAN AREAS IN SOUTHEAST BRAZIL

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Introduction: The dyes are used in the textile, pharmaceutical, food, cosmetics, photography and paper industries and products to petroleum-based. It is estimated that approximately 10%-15% of the dye used in the dyeing process is released in the environment, and may reach 50%. Environmental concern about the presence of dyes in water bodies, mostly those that will be used to capture and production of treated water for human consumption, is due that people can be exposed through ingestion of water and contaminated food. Since after ingestion, these compounds may be metabolized generating toxic products, particularly those containing azo group as a chromophore, which upon cleavage can generate mutagenic aromatic amines and/or carcinogenic. **Objectives:** This research aimed to evaluate parameters such as hydrogen potential (pH), biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, dissolved solids, oils and greases, color, turbidity, sulphate, hydrogen sulfide, dissolved oxygen, iron, phosphorus and evaluated the acute toxicity. **Materials and methods:** The methodology was applied to samples from the river in southeast Brazil. This assessment was made at a point upstream and another downstream of the discharge point of the textile effluent. The parameters were evaluated according to Rice and collaborators (2012) and the acute toxicity was evaluated using the microcrustacean *Daphnia similis*. **Results and discussion:** The results were compared with the maximum allowed value in the Brazilian legislation (CONAMA Resolution 357/2005). It was found that the parameters phosphorus, iron, hydrogen sulfide, BOD and color presented values above of allowable by legislation. In this case, the discharge of this effluent contributes significantly to increase the organic load in the receiving water body. Although COD and dissolved solid are within the limits of current regulations, these parameters showed a significant increase of the values obtained in downstream of the discharge point of the textile effluent compared with the point upstream. However, the points of river studied did not show any toxicity to *Daphnia similis*. **Conclusion:** the release of textile effluents may pose a risk to environmental quality, and consequently may affect the local population.

Acknowledgements: Universidade de Itaúna (UIT)

Reference: Rice EW, Baird RB, Clesceri AD & Lenore S. Standard Methods for the examination of water and wastewater 22ed., 2012.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 22- EVALUATION OF CHLORPYRIFOS RESIDUE, AN ORGANOPHOSPHATE PESTICIDE WIDELY USED IN BRAZIL, IN CAULIFLOWER, IN WATER AND IN SOIL

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Introduction: the increase in the development of pesticides and agricultural sector in recent years was due to the large population growth and also to increase, in the short term, the availability of food. The global market for pesticides industries grew by 93% over the past decade, while Brazilian market grew 190%. Recent studies show that organophosphates represent about 38% of the total pesticides used worldwide. A large amount of pesticide or accumulates in the soil or contaminate water resources after application. In this context, we highlight the Chlorpyrifos, an organophosphate pesticide, which is among the most widely used in Brazil and around the world. Routes of human exposure to it include: ingestion, inhalation and dermal exposure, whereas dietary exposure is the main source of non-occupational exposure from traces of Chlorpyrifos in food. It may cause toxic effects on non-target organisms, especially mammals, due to inhibition of acetylcholinesterase, which leads to accumulation of acetylcholine in nerve endings. **Objectives:** the aim of this study was to identify and to quantify Chlorpyrifos residue in cauliflower, in water and in soil after the grace period of 21 days of the last spraying this pesticide, and monitor whether this amount would be below the Maximum Residue Limit (MRL). **Materials and methods:** for this, was used the high performance liquid chromatography (HPLC) which provides the quantitative analysis a high degree of accuracy and precision in liquid or solid samples. As a reference was considered the MRL for Chlorpyrifos in vegetables cauliflower foreign literature database - *Codex Alimentarius*, 0.05 mg/kg, while the MRL of this pesticide in drinking water corresponding to 0.03 mg/L was compared to the reference value issued by World Health Organization, once in Brazil this value was not determined. **Results and discussion:** The results of the field trials performed to evaluate the persistence of Chlorpyrifos pesticide on agricultural practice demonstrated that their application to safe levels is quite commendable, considering that the study showed that the detection of this pesticide occurred at lower levels to MRL in cauliflower and water, being that the values obtained were of 0.00331 and 0.00038 mg/kg respectively and there was no detection in the soil after the time grace period of 21 days confirming that, indeed, its biodegradation is quick and its persistence is low. **Conclusion:** therefore, monitoring of pesticide residues Chlorpyrifos should be carried out constantly to prevent and reduce risks to human health and the environment, it ensures an intake of foods whose presence this pesticide is controlled and appropriate. Additionally prevents contamination of soil and groundwater located close to the plantation.

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EN 23- CITOTOXICITY AND MUTAGENICITY OF WATER SAMPLES FROM THE GUARIBAS RIVER (PICOS-PI/BRAZIL)

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Introduction: Environmental pollutants have been a major problem regarding lower fresh water quality, inducing harmful effects on living organisms that are in direct or indirect contact with them. DNA damage is one of the most frequent events caused by environmental agents. The use of Allium Cepa as a testing system is indispensable for genotoxicity analysis, since it is as reliable as animal models and cell cultures but are much more simplier. The Guaribas river is one of the major rivers of the Piauí State / Brazil, and has suffered in recent years, with intense human activities enhanced by the climatic conditions in the Northeast region of the country. **Aim**: Assessment of cytotoxicity and mutagenicity of Allium cepa meristematic roots exposed to water samples from different points of the Guaribas river, including points upstream, within and downstream the city of Picos -PI. **Materials and Methods**: Samples were collected in February / 2014 (rainy season) and September / 2014 (dry season). Five different points were analyzed: upstream the city (P1), within the city (P2 to P4) and downstream the city (P5). For cytotoxicity and mutagenicity, we used anatomical and morphological parameters (roots size-RS), mitotic index (MI), frequency of micronuclei (MN) and chromosomal abnormalities (CA). The exposure times (ET) were assessed 48, 72 and 168 hours. **Results and discussion**: Regardless of the reporting season (dry or rainy), the exposure times of 72 and 168 hours for RS and MI showed cytotoxic effects ($p < 0.05$) of water samples collected within and downstream the city when compared to the negative control. Mutagenicity, evaluated by the presence of MN and AC, showed statistically significant results for P2 ,P3 points (within the city) and P5 (downstream the city) for both seasons in the 72 and 168hs ET. Semi-arid regions of the Brazilian northeast are characterized by low rainfall thus possibly aggravating human activities in aquatic environments such as the Guaribas river. Toxickinetics and Toxicgenetics effects observed in this study might be related to the industrial and sanitary waste and pesticides released on the Guaribas river. Further analyzes on metals, hydrocarbons and cyanotoxins should be performed in order to elucidate the toxicological effects found in this study. **Conclusion**: Considering the frequency RS, MI, MN and CA, it is assumed that the water samples from the Guaribas river are mutagenic and citotoxic, since it affects meristematic roots of A. cepa in points located within and downstream the city of Picos-PI, suggesting human activities as the main source of mutagens release.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 24- GENOTOXIC EFFECTS OF WATERS OF THE POTI RIVER, BRAZIL, UNDER THE INFLUENCE OF CYANOBACTERIA

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Introduction: The occurrence of cyanobacterial blooms has significantly increased worldwide because of water eutrophication. These blooms are dangerous for humans, animals and plants, due to the production of cyanotoxins. There is evidence that certain cyanobacterial toxins are genotoxic and carcinogenic. The Poti River is a major river of the Piauí State, Brazil, which has been subjected to artificial eutrophication of its waters in recent years, thus resulting in high biovolume of cyanobacterial species in this aquatic environment. In this way, it becomes important to identify these species and to perform genotoxicological studies in this river, as it is used by the population for water supply, fishing and recreation. **Objective:** The study aimed to evaluate the genotoxicity of waters of the Poti River, Piauí State, and to identify cyanobacteria species in this aquatic environment. **Material and methods:** We evaluated three sampling sites in the Poti River inside the city of Teresina, the main city on its bank. Water samples were collected during the dry period (September 2014). Samples were analyzed for phytoplankton biovolume, using the methodology described by the American Public Health Association (APHA) and, for genotoxic analysis, we used the Comet assay in *Oreochromis niloticus*. **Results and discussion:** The results of biovolume (%) indicated the presence of the following cyanobacteria: *Planktothrix agardhii* (93.65%), *Radiocystis fernandoi* (0.18%), *Microcystis aeruginosa* (0.18%) and *Cylindrospermopsis raciborskii* (4.4%), the first three species release microcystins, responsible for increases in the production of reactive oxygen species (ROS), aneugenic activities and problems in the repair mechanism. Since the species *C. raciborskii* releases cylindrospermopsin, which causes breaks in DNA and triggers tumor initiation. According to the International Agency for Research on Cancer (IARC), both cyanotoxins are mutagenic and carcinogenic (Group 2B). For the comet assay, all the sites were statistically significant ($p < 0.05$) compared with control (local fish farming water) for the times of 24 and 48 hours of exposure. Several ecotoxicological studies show mutagenic effects caused by cyanobacterial blooms in artificially eutrophicated aquatic environments. **Conclusion:** The section of the Poti River that crosses the city of Teresina, Piauí State, Brazil, presents cyanobacterial blooms probably caused by eutrophication due to anthropogenic impacts on this environment and the waters of this river proved to be genotoxic to blood cells of the test bioindicator.

EN 25- BASIC BLUE 99 INDUCES TO CITOTOXIC EFFECTS ON HUMAN KERATINOCYTES (HaCaT)

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Introduction: The cosmetics are largely used for many years ago, in order to improve or modify the appearance. Egyptians were the first in coloring hair, using henna. Nowadays, it is estimated that 50 to 80% of the women worldwide have already dyed hair at least once. However some studies have reported toxic effects regarding on aromatic amines that can be formed during the cleavage of azo hair dyes. The Basic Blue 99 dye is used in semi-permanent non oxidatives and temporary commercial hair dyes, and the European Scientific Committee (SCCS/1537/14)[1] has recommended 1% of this compound as the maximal concentration in commercial formulations. Considering 100mL of a regular cosmetic product, this 1% represents about 10.000 µg/mL. **Objective:** In this context, we evaluate the cytotoxicity and genotoxicity of Basic Blue 99 dye in human keratinocytes (HaCaT), using doses between 10 to 50 µg/mL. **Materials and Methods:** The cytotoxicity was evaluated using Anexin V/PI protocol of BD Biosciences[2], and genotoxicity was evaluated using the protocol of Tice et al. (2000)[3] without SDS addition. We used human keratinocytes (HaCaT) cultivated in monolayer. **Results and Discussion:** Basis Blue 99 does not induce to DNA breaks in human keratinocytes (HaCaT), using the maximal dose of 20 µg/mL. However, this compound induces to an intense cytotoxicity. We observe a dose response reduction on cell viability starting at 10 µg/mL, with pronounced cell death at 50 µg/mL (70%). For the cell viability assay, we used anexin V/PI assay, however, the cell death mechanism wasn't elucidated. Because of that, we hypostasized that Basic Blue 99 induces to autophagy. **Conclusion:** The Basic Blue 99 does not induce to genotoxic effects on human keratinocytes. However, this compound could not be considering safe for cosmetic proposes considering that it induces to cell death, probably by autophagy mechanism, in doses 20 times lower than the SCCS recommendation.

References:

1. Scientific committee on consumer safety opinion on Basic blue 99 COLIPA C059. (SCCS/1537/14, 2014), http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_067.pdf. Acessed in 05.03.2015.
2. Anexin V Staining Protocol (BD Biosciences, 2015), http://www.bdbiosciences.com/br/resources/protocols/annexin.jsp. Acessed in 14.07.2015.
3. R.R.Tice *et al*. *Env. Mol. Mut.*35:206, 2000.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 26- MONITORING AND GENOTOXICITY OF PB, HG, AS AND RELATIONSHIP TO BIO-SOCIAL CONTEXT IN CHILDREN FROM YUCATAN COAST, MEXICO.

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Introduction: Lead (Pb), Mercury (Hg) and Arsenic (As) are among the ten most toxic chemical agents according to the WHO. Genotoxic effects have been associated with Pb, Hg and As exposure. Children are an especially vulnerable population, particularly in developing countries communities were environmental and sanitary conditions are not optimal and low educative level in addition to living conditions increase the risk of health problems. Thus, monitoring the levels of metals and detection of early effects in children are very important to avoid irreversible health outcomes. **Objective:** To determinate Pb, Hg and As and to evaluate the frequency of micronuclei and nuclear atypia in epithelial cells, and to explore their relationship with the bio-social context of children from a coastal locality of Yucatan, Mexico. **Material and Methods:** This transversal study involved children between 7 and 9 years of age who attend public schools. For the identification of possible sources of metals and bio-social context, questionnaires and semi-structured interviews they were conducted. Pb in blood (Pb-B), Pb urine (Pb-U), Pb in consumed water (Pb-W), Hg in blood (Hg-B), Hg in (Hg-U), Hg in consumed water (Hg-W) were determined by HGAAS. As in blood (As-B), urine (As-U) and consumed water (As-W) were determined steam cold. The genotoxic effects were evaluated using micronucleus cytome assays in buccal epithelial cells. **Results and discussion:** A total of 35 children participated in the study. Lower-middle socioeconomic class households predominated in the sample. The mean concentrations of metals in blood and urine (µ/dL) in children and consumed water (ppb) did not exceed the reference values (RV). However, 17% of the study population had levels of Hg-U above the RV and 89% of children exhibited the presence of metals in their bodies. Pb was detected in 31% of drinking water samples, but only in 6% of the cooking water samples. Sixty-nine percent of drinking water samples and 80% of the cooking water samples had Hg. As was present in 89% of the drinking water samples and 80% of cooking water samples. MN/1000 cells and atypias/1000 cells frequency were not above the RV, only binucleated cells frequency was above the RV in 26% of children. Picnosis frequency was related with As-U levels ($p<0.01$) and the broken egg frequency was related with Pb-B ($p= 0.06$). **Conclusions:** Our monitoring program represents the first conducted in children from this region and our results suggest cytotoxic and genotoxic effects in buccal epithelium of children. We suggest continued monitoring to avoid irreversible health damage in children.

Acknowldgements: Thanks to Conacyt for providing financial support for this project.

EN 27- RISK-BASED METHODOLOGY FOR SOIL QUALITY MANAGEMENT: THE CASE OF INORGANIC ARSENIC IN THE CITY OF ARICA, CHILE.

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Introduction: Soils in the city of Arica, Chile, naturally contain high concentrations of arsenic and metals. In addition, open storage of waste materials containing elevated concentrations of As, Cd, Cr, Hg and Pb among other industrial activities have increased the level of these contaminants in the city. A decline in soil quality eventually worsens the living conditions of its population. Remediation and restoration of degraded soil is therefore necessary to protect human health and to maintain its quality of life. **Objective:** The aim of this work was to assess the risk to human health due to current levels of contaminants in soil and to determine a maximum acceptable concentration (MAC) in this matrix for contaminants exceeding acceptable risk as a quality guideline for risk management. **Materials and Methods:** A human health risk assessment was conducted in order to determine potential impact of As, Cd, Cr, Hg and Pb exposure on the population. Exposure was derived using measurements from soil and air from representative samples throughout the city. To characterise risk, exposure by each route was contrasted to minimal risk levels for chronic non-carcinogenic effects, when available. Hazard ratios (HR) and hazard index (HI) < 1 were deemed acceptable. The lineal model was used to determine incremental cancer risk from lifetime exposure to carcinogens. Carcinogenic risk was deemed unacceptable if greater than 1E-6. For contaminants exceeding carcinogenic and/or non-carcinogenic effects risk a MAC in soil was derived as a guideline tool for soil quality management. This maximum concentration was calculated based on acceptable risk over background concentration of the contaminant. **Results and Discussion:** None of the contaminants exceeded the acceptable risk for non-carcinogenic effects. Exposure to arsenic exceeds the acceptable risk for carcinogenic effects over a lifetime. A MAC was therefore derived for inorganic arsenic. This MAC represents a site-specific quality guideline value for risk management and land planning. It may represent an initial methodological approximation for the development of regulatory guidelines values currently non-existent in the country. Of note, this MAC takes into account background concentration of arsenic in soil. This is due to the fact that arsenic guideline values based solely on risk are usually lower than background levels posing a challenge in terms of risk management and communication. **Conclusions:** Current exposure to arsenic represents an unacceptable risk to human health in the city of Arica. A MAC for inorganic arsenic is proposed as quality guideline for soil risk management. Methodology followed for MAC calculation may be considered as an approximation for regulatory guidelines values in Chile.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 28- NEONATAL EXPOSURE TO A GLYPHOSATE BASED HERBICIDE ALTERS THE DEVELOPMENT OF THE RAT UTERUS

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Introduction: Glyphosate-based herbicides (GBH) are extensively used to control weeds on both cropland and non -cropland areas. *In vitro* and *in vivo* studies have reported endocrine disrupting effects and male reproductive toxicity at puberty and adulthood caused by GBH exposure. However no reports are available regarding its effects on the development and performance of the female reproductive tract. **Objective:** To evaluate the effects of neonatal exposure to a GBH on uterine morphology, proliferation and expression of proteins that regulate uterine organogenetic differentiation in rats. **Material and Methods:** Female Wistar pups received saline solution (control, C) or an environmental relevant dose of commercial formulation of glyphosate (GBH, 2 mg/kg) by sc injection every 48 h from postnatal day (PND) 1 to PND7. Rats were sacrificed on PND8 (neonatal period) and PND21 (prepubertal period) to evaluate acute and short-term effects, respectively. The uterine histomorphology was evaluated in hematoxylin and eosin stained sections. The epithelial and stromal immunophenotypes were established by assessing the expression of luminal epithelial protein (cytokeratin 8; K8), stratified epithelial proteins (p63 and pan cytokeratin -K1, -K5, -K10 and -K14); and vimentin (a cytoskeletal protein expressed in mesenchymally-derived cells) by immunohistochemistry (IHC). The uterine cell proliferation was detected by the expression of Ki-67 protein. In addition, we evaluated the expression of estrogen receptor alpha (ERa), progesterone receptor (PR) and Hoxa10 by IHC in all uterine compartments (LE: luminal epithelium, GE: glandular epithelium, SS: subepithelial stroma, M: myometrium), to investigate changes in proteins that regulate uterine organogenetic differentiation. **Results and Discussion:** The GBH-exposed uteri showed morphological changes. The most relevant change was the luminal epithelial hyperplasia (75% of animals on PND8 and 37.5% on PND21). The epithelial cells showed a positive immunostaining for K8 and the stromal cells for vimentin. GBH-treated group showed an increase cellular proliferation in the LE and SS on PND8, without changes on PND21. In addition, the uterine organogenetic differentiation was affected at both ages. An induction of PR and Hoxa10 was detected in all cellular compartments of GBH-treated rats on PND8 and ERa was also up regulated in the SS. The deregulation of PR and Hoxa10 persisted on PND21. **Conclusions:** Neonatal exposure to GBH disrupts the postnatal uterine development and alters the expression of proteins involved in uterine organogenetic differentiation at the prepubertal period. All these changes may alter the functional differentiation of the uterus, affecting the female fertility and/or promoting the development of neoplasias.

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EN 29- ENDOSULFAN AFFECTS UTERINE DEVELOPMENT AND FUNCTIONAL DIFFERENTIATION BY DISRUPTING WNT7A/B-CATENIN SIGNALING PATHWAY

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Introduction: The exposure to endocrine disruptors in early period of development may generate long-term effects in female reproductive tract. In a recent work we showed that neonatal exposure to low doses of the widely used endosulfan pesticide, classified as xenoestrogen, decreases the pregnancy rate and the number of implantation sites in rats. Wnt7a/β-catenin signaling is critical for proper uterine development during embryogenesis and gland formation. This pathway also plays a key role in regulating gland functional differentiation at adulthood, a process that is crucial for endometrial receptivity. Failures in this pathway may compromise embryo implantation and fertility. **Objective:** To investigate if endosulfan-implantation failures are associated with an impaired gland formation and/or a deregulated Wnt7a/β-catenin signaling in prepubertal and adult female rats. **Material and Methods:** Newborn female Wistar rats were treated by sc injections with vehicle (control, C), endosulfan (600 ug/kg b.w /d, E600) or diethylstilbestrol (0.2 µg/kg b.w./d, DES, used as an endocrine disruptor control) on PND (postnatal day) 1, 3, 5 and 7. Female rats were sacrificed on PND8 (neonatal period) and PND21 (prepubertal period) to evaluate the acute and short-term responses, respectively; and at the pre-implantation period (gestational day 5, GD5), to evaluate the long term effects on the uterine functional differentiation. At these three time points we determined the expression of Wnt7a and β-catenin proteins in uterine sections by immunohistochemistry. On PND21 and GD5 we also quantified the number of endometrial glands. **Results and Discussion:** E600 group showed an increase of Wnt7a and β-catenin proteins in the epithelium on PND8 and a decrease of Wnt7a in the glands on PND21. Although no changes in gland number were observed on PND21, a lower number of uterine glands were recorded in E600 and DES groups on GD5. In addition, we observed a decrease of Wnt7a expression in all uterine compartments and an increase of β-catenin expression in the luminal and glandular epithelial cells of E600- and DES-exposed rats on GD5. The early exposure to endosulfan deregulates the uterine expression of both Wnt7a and β-catenin in neonatal and prepubertal female rats and these alterations persist at adulthood. In the pre-implantation period, the deregulation of Wnt7a/β-catenin signaling was associated with a decrease in the number of uterine glands. **Conclusions:** The disruption of the uterine Wnt7a/β-catenin signaling in prepubertal and adult females may be involved in the implantation failures and subfertility triggered by early postnatal endosulfan exposure.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 30- ENVIRONMENTAL EXPOSURE TO MERCURY FROMA CONTAMINATED SOIL IN DESCOBERTO, MG

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Introduction: Mercury (Hg) is a neurotoxic metal, widely spread in the environment.Large amounts of alluvial gold were taken from Descoberto (MG, Brazil) in the past and Hg was buried in the soil after use. Later, Hg emerged from the ground when a road was opened. **Objective:** The aim was to assess levels of mercury in air (Hg-A), soil (Hg-S), water (Hg-W), urine (Hg-U) and hair (Hg-H) of the population. **Materials and Methods:** Hair and soil were kept in plastic bags, urine and water collected in containers, and air was sampled by portable equipment, Lumex, also used to determine mercury concentration in all samples by cold vapor atomic absorption spectrometry. **Results and Discussion:** Number of participants was 94.Men were 52% (49) and 48% (45) women. Mean age was 39.0± 24.2 years, with men (43.8 ± 24.3) 10 years older than women (33.9 ± 23.3). Median for Hg-U was 0.23 µg $L^{-1}$and the concentration ranged from ≤ 0.006 µg L^{-1} to 1.76 µg L^{-1}. Minimum and maximum levels of Hg-H were ≤ 0.096 and 1.92 µg g^{-1},respectively. Median found was 0.15 µg g^{-1}. After stratification by gender and age, the age group presenting the highest median for urine (0.12 µg L^{-1}) was 11-20 years for women, while those aged between 31 and 40 showed the highest Hg-H (median: 0.24 µg g^{-1}). Concerning men, the highest median was 0.18 µg L^{-1} for urine in the age group from 51 to 60 years. The elderly, between 71 and 80, showed the highest level of Hg-H (median: 0.26 µg g^{-1}).The number of air, soil and water samples was 43, 21 and 33, respectively. The median of Hg-A was 27.8 ng m^{-3}. The area flowing Hg from the soil showed levels ranging from 55-105 ng m^{-3}. However, other three regions had higher concentrations than those found in the known contaminated area. One of them was the site for water catchment (132 ng m^{-3}) of the town and the other two were near a dam (138.6 ng m^{-3}) and lagoon (112 ng m^{-3}). Soil presented a median of 0.22 mg Kg^{-1}, a minimum value of 0.083 mg Kg^{-1} and the maximum of 3.43 mg Kg^{-1}in the contaminated area. Regarding water samples, 81% (27) of them were below ≤ 0.90 ng L^{-1}. However, Hg was present in other 6 samples from relevant areas such as water supply and treatment, varying from 1.1 to 2.8 ng L^{-1}. Although Hg-W were below the reference values, air samples showed concentrations higher than those found in contaminated regions of the Amazon (from 1.3 to 1.6 ng m^{-3}) and urban areas (3.6 ng m^{-3}) with gold trade. Likewise, levels similar to those existing in soil from mining sites in the Amazon (0.03 to 0.37 mg Kg^{-1}) and urban areas (0.030 to 1.33 mg Kg^{-1}) next to gold trade shops were discovered. **Conclusion:** Such data associated to clinical and neuropsychological evaluation point out to the need of surveillance actions and remediation of the contaminated site.

EN 31- LEVELS OF TIN IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES IN THE VICINITY OF AN ALLOY INDUSTRY

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Introduction: Tin (Sn) is widely used in industry. Welding and bronze are the major tin alloys currently used. Tin accumulates in bones and may produce different effects on neurological, blood and immune systems, as well as damage to the liver and kidneys. The literature on its effects in humans is scarce, especially with regard to reference values in biological indicators such as blood and urine. Similarly, environmental limits are also rare. **Objective:**The aim of this study was to assess the level of the environmental exposure to tin in the vicinity of an alloy industry located in Volta Redonda, RJ. **Materials and Methods:**The study population consisted of 74 adults.A questionnaire has requested information about socioeconomic data and investigated potential confounders as well as signs and symptoms consistent with tin contamination. Electrothermal atomic absorption spectrometry was used for the determination of tin in environmental and biological samples. The atmospheric air was collected for a period of 5 hours using a medium volume sampler with a flow rate equal to 20L min^{-1}. A portable air sampling pump collected the household dust, with flow rate calibrated to 2.0 L min^{-1}. Air sampling was carried out in 18 points during two visits, assuming the tin alloy industry as a stationary source. A total of 8 samples of household dust were collected in both campaigns. Whole blood was collected in heparinized vacutainer tubes for trace analysis, whereas urine was collected in 50 mL containers previously decontaminated. **Results and Discussion:** The results of the first and second campaigns ranged from 0.022 to 0.153 and 0.003 to 0.445 µg m^{-3} for the atmospheric air, whereas such ranges were 0.64 to 1.61 and 1.97 to 8.54 µg m^{-2} for household dust, respectively. Air concentrations of tin in US cities fromseveral studies were as high as 0.8 µgm^{-3} withaverageconcentrations generally below 0.1 µgm^{-3}. The average tin concentration found in blood of the population (n = 65 individuals) was 3.85 ± 1.57µg L^{-1}. Regarding urine (n = 68 participants), such value was 3.56 ± 1.88 µg L^{-1}. In a previous study, the authors analyzedblood and urine samples from eleven volunteers not occupationally exposed to tin. Levels of tin in blood ranged from 7.4 to 11.2 µg L^{-1} while urine concentrations varied from ≤ 0.8 to 2.2 µg L^{-1}. **Conclusion:** Environmental monitoring becomes necessary to better assess the exposure to tin since it is an industrial region with a high environmental exposure to metals.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 32- EVALUATION OF CYTOTOXICITY AND GENOTOXITY OF PRINTING INDUSTRY WASTEWATER SUBMITTED TO TREATMENT WITH CLINOPTILOITE ZEOLITE

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Introduction: The printing industry is a significant source of water pollution, in view of the fact that printing process produces wastewater containing pollutants that are extremely harmful to people and the environment. Therefore, it is necessary to elaborate efficient treatments that permit the suitable reuse and liberation of this wastewater into the environment. Natural Zeolite has been widely used as an absorbent in wastewater separation and purification processes, mainly due to its great cationic exchange capacity, molecular sieve, high availability and low cost. **Objective:** Considering the potential application of Zeolite in the treatment of wastewater, we decided to investigate the citotoxicity and genotoxicity of printing industry wastewater submitted to treatment with Zeolite. **Materials and Methods:** *Allium cepa* seeds have been exposed to printing industry wastewater treated with Zeolite at different concentrations: 0.5 g/L, 1.5 g/L, 3.0 g/L, and 10 g/L. The negative control group has been exposed to ultrapure water. Also, some *Allium cepa* seeds were treated with raw effluent. All seed were exposed during 96 hours. Subsequently, the germinated roots have been measured, fixed into methanol acetic acid (3:1) for 24 hours and kept into alcohol 70% for citotoxicity and genotoxicity tests. A number of 12000 cells have been analyzed to each tested concentration. The analysis of slides consisted of obtaining the mitotic index (MI) and chromosomal aberrations (CA) for all tested groups. The statistic analysis was performed using the GraphPad Prism 6.0 Program. **Results and Discussion:** Concerning cytotoxic parameters, our results showed that raw effluent induced damage effects, inhibiting seeds germination. On the other hand, the wastewaters that were treated with Zeolite did not induced cytotoxic effects. Concerning genotoxic parameters, the raw effluent and the wastewaters that were treated with Zeolite at 0.5 g/L, 1.5 g/L, and 3.0 g/L induced chromosomal aberrations. Nevertheless, the wastewater treatment with Zeolite at 10 g/L was able to prevent the induction of chromosomal aberrations. Therefore, our results indicate that Zeolite is effective in order to decontaminate wastewater from industrial printing. **Conclusions:** Our results have shown that Zeolite could be an efficient tool in aim of turning wastewater from printing industry without genotoxic and cytotoxic effects. We suggest that Zeolite can be used as an alternative product because of its high availability, easy handling, low cost, and selectivity.

EN 33- ANALYSIS OF THE QUALITY OF THE RIVER ITAPEMIRIM / ES USING *Allium cepa* L. BULB AS BIOASSAY

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Introduction: The Itapemirim river is considered one of the most important water resources of Espírito Santo. However, environmental problems may be affecting the Itapemirim river, that can be caused by disorderly occupation of their surroundings, by wrong utilization of pesticides in agriculture or in stone sector os Espírito Santo. Given of the ecological and socio-economic importance of water resources, the preoccupation with the water qualitty is crucial. **Objective:** This study aims evaluate the water quality of Itapemirim river, through cytogenetic analysis, using as test organism the *Allium cepa.* **Materials and Methods:** They were defined 4 sampling stations to collect subsurface water along of Itapemirim river's path (PRI01, PRI02, PRI03 and PRI04). The *Allium cepa* bulbs were added in test tubes containing the water samples collected in the sampling stations and of positive ($4x10^{-4}$ mol/L of Methyl Methane Sulfonate) and negative (distilled water) controls. Rootlets abaout 1 cm were collected and fixed in carnoy (3 : 1). For cytogenetic analysis, blades were made from meristem regions, and subsequently stained by the Feulgen method. After obtaining the results, were made the statistical test utilizing the free software Genes (CRUZ, 2008), using analysis of variance and Tukey test at 5% probability. **Results and Discussion:** The changes found were micronucleus, C-metaphase chromosome sticky, bridge and delay. Among changes the ones that present the highest frequency were C-metaphase and micronucleus. The point 2 was what present the highest number of statistically different changes compared to the negative control and other points, and is the one that had the highest IM (Mitotic Index), demonstrating disorderly cell proliferation and consequently causes the formation of abnormal individual. Although not observed significant difference between treatments for some of the variables analyzed, generally, the points 2 – 3 – 1 e 4, respectively, they showed an extender in the amount of changes. Probably this increase is related to the fact that the points 2 and 3 stay near urban area of city of Cachoeiro de Itapemirim and of processing industries of marble and granite slabs, main economic sector in the region, whereas point 1 is nearby the small district of Pacotuba and point 4 be further down the urban area. **Conclusion:** The results found indicate that the stretches along the Itapemirim river basin present cytotoxic, genotoxic and mutagenic effect, indicating a strong anthropogenic influence in this environment, especially at points upstream of the urban area of the city of Cachoeiro de Itapemirim/ES.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 34- PHOTOSYNTHETIC AND CYTOGENETIC RESPONSES OF PLANTS AFTER EXPOSURE TO A IMPACTED ENVIRONMENTAL POND

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Introduction: Plants are sensitive to environmental pollutants, being great organisms for genetic and physiological tests. The test systems *Allium cepa* and *Tradescantia pallida*, are used to study of water, air and land pollution. Among the biomarkers, cytogenetic damage and chlorophyll content are used in stress studies due to their answers to single or combined factors. The Juara pond is one of the most important water bodies in the metropolitan area of Vitória/ES, presenting important ecological and socio-economic function, being used to leisure, recreation, sport fishing and intensive fish farming in tanks networks. **Objective:** Thus, this study aimed assess the photosynthetic responses in *T. pallida*, and cytogenetic in *A. cepa* exposed to water of three sampling stations of Juara Lagoon (Municipality of Serra/ES), collected in two sampling campaigns. **Materials and Methods:** From shallow pond water samples, the tests were conducted with plants. In the first, chloroplastid pigment levels were measured in leaves of *T. pallida* after chronic exposure of their stakes. In the second, the cytotoxic, genotoxic and mutagenic potential were analyzed by *Allium cepa* of test solutions in the tests with *T. pallida.* For statistical analysis of metals was used Kruskal-Wallis test ($p < 0.05$) and to biotic data, ANOVA followed by Tukey test ($p < 0.05$). **Results and Discussion:** The study of photosynthetic metabolism in *T. pallida* showed that the pigment contents are related to the high supply of nutrients present in the stations. Being overweight these, the probable responsible for the level pigments in the lower one. Already cytogenetic tests *A. cepa* demonstrated that two sampling stations in at least one sample campaign showed cytotoxic, genotoxic and mutagenic potential. This suggests the presence of potential pollutants, in view of the launch of domestic sewage in the river basin of Juara pond. **Conclusion:** The excess of nutrients appears to have toxicity on photosynthetic metabolism of *T. pallida*, whereas the *A. cepa* cytogenetic damage may have been caused by other pollutants such as herbicides and domestic effluents. Therefore, the use of both tests are presented in interesting and complementary evaluation Juara pond.

EN 35- PHYTOTOXICITY AND GENOTOXICITY OF TEXTILE SLUDGE AQUEOUS EXTRACTS TO *Allium cepa* L.

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Introduction: The textile industry consumes large quantities of water and hence generate and release large volumes of effluent which, when treated by biological systems generate large amounts of sludge. This sludge is a mixture of organic and inorganic compounds, including dyes and metals, which can be hazardous due to of its toxicity. **Objective:** The objective of this work was to assess the phytotoxicity and genotoxicity of two samples of textile sludge using *Allium cepa* L. **Material and methods:** Two samples of sludge (August and October of 2014) were collected from a textile industry located in Minas Gerais State. The samples were dehydrated, pulverize and sifted. The aqueous extractions were performed with distilled water (1:4 w/v), stirring for 24 h. Both samples extracts were tested at 20, 40, 60 80 and 100%. Distilled water and $ZnSO_4$ (0.07 g L^{-1}) were used as negative and positive controls. The tests were performed with 30 seeds per plate, in triplicates. The phytotoxicity endpoints evaluated were the percentage of germination (G%), root elongation (RA), and fresh biomass (FB) production. The 50 % inhibition concentrations (IC_{50}) were calculated by linear interpolation, available in ICP_{IN} software. For genotoxicity the endpoints evaluated were mitotic index (MI), and chromosome aberrations (CA). 3000 cells were evaluated for each tested concentration. Data were submitted to analysis of variance (ANOVA) (p\0.05) followed by Scott-Knott test ($p > 0.05$) using Sisvar software. **Results and discussion:** The first sample, collected in August, was the most phytotoxic with IC_{50} of 60% and 12% to G% and RA, respectively. The second sample was nontoxic for G% and presented an IC_{50} of 98% for RA. Both samples were nontoxic for the endpoint FB. The most sensitive phytotoxicity endpoint was RA, although the second sample was only slightly toxic. The MIs were reduced for the first samples from the concentration of 60% (7.7%) and for the second sample from the concentration of 40% (10.7%). The MI results showed the cytotoxic effect of the extracts; however both extracts were non genotoxic. None of the tested concentrations were able to induce increase in CA frequency. **Conclusion:** Despite the wastes produced by textile industries are considered of high environmental concern, the second sample could be considered almost nontoxic. For the first sample relatively high phytotoxicity was observed only for the endpoint RA. The extracts were non genotoxic for the. The differences observed in the results for the two samplings may be related to the use of different chemical products in the industrial process. So new samplings will be performed and other endpoints will be included, both for phytotoxicity and genotoxicity evaluations.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 36- GENOTOXICITY AND ECOTOXICITY IN ZEBRAFISH EMBRYOS AS TOOLS IN ENVIRONMENTAL MONITORING PROGRAMS – PRELIMINARY RESULTS

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The replacement of animal tests for ethical and regulatory reasons have brought the attention to fish embryos, since their use is not regulated by current legislations on animal welfare. An alternative approach to classical acute fish toxicity testing of chemicals is the fish embryo toxicity (FET) test, which has been standardized at the international level. The possibility of combining ecotoxicological and genotoxicity tests on the same organisms exposed is also thrilling. The comet assay is a sensitive and simple technique to assess the genotoxicity of complex environmental mixtures, and it is increasingly being used in environmental biomonitoring. The aim of the study was to investigate the applicability of the FET test combined with the Comet Assay on the detection of toxic and genotoxic effects of zebrafish embryos exposed to chemical samples, surface water and effluents. The zebrafish embryos collected after 2hpf were exposed for 96h to 6 concentrations of $ZnCl_2$, 3 surface water samples and 7 concentrations of 2 industrial effluents. For the FET test, embryos were checked after 96h to verify coagulation of embryos, lack of somite formation, non-detachment of the tail and lack of heartbeat. LC50:96h values were calculated for $ZnCL_2$ and effluent samples. After the final exposure time, the surviving embryos were tested for genotoxicity. Embryos were minced with a glass tissue grinder with a pestle. The cell suspension was mixed with low melting point agarose 0.7%, lysed, and electrophoresis was proceeded in alkaline buffer, at 25V/300mA for 25 minutes. Slides were stained with 75µl of Gel Red® and tail moments were scored at a fluorescent microscope equipped with the Comet Imager 2.2 software (MetaSystems). No toxic effects were observed for the environmental samples analyzed. LC50:96h calculated to $ZnCl_2$ was 60.5 mg/L. No genotoxic activity was observed to the effluent samples, and despite that there was no significant difference between control and the surface water samples and $ZnCl_2$, we observed a double fold induction of tail moment for surface water sample 2 and for Zinc chloride (160 mg/L). The comparison between tail moment control values of all experiments, showed a significant difference ($p<0.05$). This could be due to the maceration process that could increase DNA-fragmentation and distort the results. Preliminary results of the combination of toxicity and genotoxicity endpoints in zebrafish embryos exposed to different samples, although in need of methodological improvement, showed a promising strategy to be used in monitoring programs. The more interesting fact being the possibility of the observation of two endpoints in the same organism exposed.

EN 37- LEVELS MERCURIAL EXHIBITION IN CHILDREN AND TEENS THE TWO CITIES OF THE PARÁ STATE, IMPACTED BY INDUSTRIAL PROCESSES

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Introduction: Different social vulnerability levels can affect populations in the vicinity of industries in terms of environmental risks. Adjacent to the industrial complex of Barcarena, has been identified increased vulnerability of people living near the river in relation to mainland residents. The spread of pollutants from these sources can reach varying distances through air, water, soil and sediment and biota arrive, allowing the exposure of communities to contaminants. Children and adolescents, age marked by significant biological changes that can be changed by external and internal risk factors, leading to changes in body homeostasis, may suffer various kinds of diseases.External risks such as heavy metals especially as mercury (Hg) are the most dangerous to children's environmental health. **Objective**: This study was part of an Environmental Monitoring Program developed by the Evandro Chagas Institute / SVS-MS including Human Health Assessment that this group evaluated the exposure to Hg in whole blood. **Materials and Methods**: A total of 527 individuals were surveyed aged 06-19 years communities in the City of Abaetetuba (Maranhão Village, n = 139 and Beja Village, n = 93) and Barcarena (Industrial District n = 132 and Conde Village, n = 163) in the state of Pará. Epidemiological questionnaire was applied. For Hg analysis proceeded to test the Mercury Analyzer(CVAAS), Hg201 (KK Sanso Corp., Japan).The central tendency and dispersion and dispersion meansures was used to described the population and the statistical analysis was performed using the Kruskal-Wallis ANOVA and Mann-Whitney test ($\alpha = 0.05$). **Results and Discussion**: The mean of Hg levels (µg/L) had the following values: 9.4; 4.9; 3.6 and 2.6 in Vila Maranhão, Beja Village, Industrial District and Conde Village, respectively. The level of exposure in Maranhão Village was statistically different from the other locations ($p <0.05$). Hg levels were statistically associated with fish consumption($p <0.01$).Hg levels equal or above the Reference Values (8mg / L) are reported in studies conducted in the Amazon, performed in mining areas, even in areas without anthropogenic action, demonstrating that even in remote areas of these activities, Hg may be available to the community through the food chain. Our findings corroborate the data described, highlighting average Hg 9.4µg/L in the subjects of Maranhão Village. **Conclusion**: Knowledge of the levels of exposure to metals such as Hg and forms of exposure are important health protection instruments, especially children and adolescents, given their vulnerable condition and the recognized Hg's ability to cause risk of adverse effects health at this stage.

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Keywords: Mercury, Children, Adolescents, vulnerability, Exposure.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EN 38- APPLICATION OF ENVIRONMENTAL MEDIA EVALUATION GUIDES FOR PRIORITIZATION OF SITES POTENTIALLY CONTAMINATED AFTER A NATURAL DISASTER.

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Introduction: Natural disasters may significantly contribute to human exposure to hazardous chemicals and contaminants. These events can trigger technological malfunctions leading to an unintentional release of these substances, mobilisation of stored chemicals as well as affecting mine tailings located near residential communities. Heavy rainfall and consequent flash floods and landslides affected the Atacama Desert over 24-25 March, 2015. This situation raised concern on health authorities for the potential risk to human health due to exposure to chemicals and debris from abandoned mine tailings. **Objective:** The aim of this work was to develop a set of indicators to prioritise intervention areas potentially affected by chemical contaminants in an area affected by a flood and landslides. **Materials and Methods:** An environmental media evaluation guide (EMEG) was derived for each chemical of concern identified by the Ministry of Health. EMEGs for soil ingestion were calculated using minimal risk levels (MRL) for intermediate exposure, oral route, as published by the Agency for Toxic Substances & Disease Registry (ATSDR). Children were assumed as the most exposed population. Exposure factors were those used by ATSDR except daily soil ingestion which was doubled to account for uncertainty due to extrapolation to local population. In the case of lead, due to the fact that no MRL has been derived, value from US 40 CFR Part 175 was used as EMEG. **Results and Discussion:** EMEGs were derived for As, Cd, Cr (VI), Sn, Pb, V and Zn. This set of indicators are intended to provide a quick guidance for health authorities to prioritise interventions considering an intermediate exposure (14-364 days) to potentially contaminated mud. Application of regulatory guidance values for soil quality was deemed inappropriate due to the fact that chronic or carcinogenic studies are used as point of departure for their derivation. These EMEGs are based on estimates of the daily human exposure to these hazardous substances that is likely to be without appreciable risk of adverse non-cancer health effects. Usually cancer risk estimates are based on chronic exposures. There is great uncertainty in extrapolating results from lifetime exposures to risk associated with acute and intermediate exposure, therefore, it was not considered a critical parameter for health intervention. Chemicals found below their EMEG are not expected to pose a public health hazard while chemicals found above their EMEG should prompt a prioritization for mud removal or in-depth health risk evaluation. **Conclusions:** A set of indicators was developed as a screening tool to prioritise public health interventions in sites affected by landslides and potentially contaminated.

EN 39- MOLECULAR CHARACTERIZATION OF *C.ELEGANS* RESPONSE TO GLYPHOSATE

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Introduction: After maize, Soybean constitutes the second larger crop around the world and almost 80% of this crop worldwide is genetically modified. The glyphosate resistant soybean genotype is the most common transgenic variant cultivated and in consequence, glyphosate is one of the world larger pesticides applied to crops. Most of the current literature on the toxicity of glyphosate comes from classical toxicology approaches, which focus its endpoint in viability, reproduction and fecundity but little information is known about the intracellular molecular mechanism of glyphosate toxicity in not target species. The nematode *Caenorhabditis elegans* is emerging as a valuable in vivo model, for both mechanistic and environmental toxicology, to predict outcomes in higher eukaryotes toxicity. **Objective:** The aim of this study is to establish the effect of glyphosate on gene expression of oxidative stress pathways in C. elegans in order to identify candidate genes to evaluate molecular changes in environmental water samples contaminated with glyphosate. **Materials and Methods:** *C. elegans* was originally obtained from the Caenorhabditis Genetics Center (CGC) and maintained as stocks. The nematode bioassay was carried out, with a few modifications, according to standard methods (ISO, 2010). Real time PRC and catalase assay were performed with standards protocols. **Results and Discussion:** *C. elegans* growth as well as reproduction and fertility were inhibited by treatment with commercial formulate glyphosate (Glyphosate F) in a dose-dependent manner (EC_{50} values: 1,1; 0,8; 0,8 mg/ml respectively). Upon treatment with Glyphosate F we observed an increase in the Reactive Oxygen Species (ROS) formation (similar to levels induced by Paraquat), revealing a modification in the redox balance of the organism induced by this pesticide. *C. elegans* has different molecular mechanisms that can be induced in response to increasing ROS concentration. To identify which pathways were predominantly upregulated upon exposure to glyphosate F we measure changes in the expression levels of different detoxifying enzymes. We observed a specific induction of Catalase genes (ctl-1, ctl-3 but not ctl-2) and concomitantly increase in the catalase activity. **Conclusions:** In this work we analysed the *C. elegans* response to commercial formulate glyphosate treatments. We established the EC_{50} for growth, reproduction and fertility. In addition we observed significant increase in the catalase genes expression and also their activity upon glyphosate F treatment in *C. elegans*. At the moment we are performing experiments with environmental water samples containing glyphosate in order to establish glyphosate contamination detecting protocols based on catalase activity detection.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EN 40- HEXAVALENT CHROMIUM - INDUCED TOXICITY IN *Drosophila melanogaster*

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Introduction: Heavy metals are non-degradable and can accumulate into the living tissues along the trophic chain, affecting human, especially through feeding. The Hexavalent Chromium (Cr VI) is not normally found in the nature; so its presence indicates potential anthropic impacts due to its usage in the timber, metal mechanic and tanning industries, among others. Cr VI has great potential of oxidation and easy access to cells. This element can alter the cellular structures as well as affect or substitute the enzymatic activities. **Objective:** The major objective were to evaluate the toxicity of Cr VI over behavioral, survival and biochemical parameters in *Drosophila melanogaster*. **Materials and Methods:** Flies (*Drosophila melanogaster)* have been exposed to the following concentrations of Cr VI: 2.5 mg/lt, 5 mg/lt and 10 mg/lt. Also, a group of flies were not exposed to Cr VI (negative control group). These parameters have been evaluated: survival, negative geotaxis and Acetylcholinesterase enzyme (AchE) activity. The flies have been exposed to Cr VI for 14 days.The survival rate was analyzed during the 14 days of treatment. The negative geotaxis and the enzyme activity have been analyzed in the fifth day of treatment. The results have been analyzed through ONE-WAY ANOVA, followed by the *post hoc* Dunnet. **Results and Discussion:** Our results have shown that exposure to Cr VI causes an increase of mortality that is evident from the fifth day of exposure. Regarding to the behavioral parameters (negative geotaxis), it was possible to observe that exposure to Cr VI at 10 mg/lt has significantly decreased the percentage of ascension of individuals when compared to the group of control. Considering the analysis of AchE enzyme, the exposure to Cr VI at 10 mg/lt has increased the enzymatic activity. **Conclusions:** Our results indicate that Cr VI is toxic in certain conditions, altering the behavioral, survival and biochemical parameters in the studied model. From that, it is suggested the Cr VI should receive more attention since it is an environmental pollutant that shows potential toxicity over organisms.

EN 41- CHLORPYRIFOS RISK ASSESSMENT FOR AQUATIC BIOTA AND EXCEEDENCE PROBABILITY IN TRES ARROYOS-CLAROMECO (ARGENTINA) BASIN

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Buenos Aires Province (Argentina) has undergone in the last decades a great increase in agricultural activities based on new technologies, with a reduction of cultivar diversity. I.e., in Buenos Aires the area covered with soybean was 5.9 million Ha during the cycle 2010-2011. Chlorpyrifos (CPF) is one of the insecticides most widely used in these crops and may constitute a risk for human health, birds, and aquatic biota such as macroinvertebrates and fishes. The **objective** of this study was to assess the risk derived from CPF use in aquatic life establishing P5-P10 percentiles for acute and chronic effects, to compare these endpoints with current aquatic life protection criteria, and to determine probabilities of exceedence of all these values considering CPF concentrations in water and sediment at Tres Arroyos-Claromecó basin. CPF contents found in water and sediment samples obtained in 4 campaigns at different seasons in 4 streams of the basin were used to perform exceedence risk assessment. Reference CPF data for 40 (acute toxicity) and 28 (chronic effects) aquatic species were used to perform a Risk Assessment probit analysis and establish P5 and P10 values (CPF concentrations affecting 5% or 10% of total species respectively). Environmental CPF data at the local basin were also ordered in a percentile distribution, and a probit model was fitted to data to obtain the probabilities of exceedence respect to risk endpoints (Joint-Probability curves). Aquatic life protection criteria from Canada (long term exposure: 0.002 µg/L; CCME, 2008) and the Water Resources of Argentina (0.006 µg/L; 2005) were introduced in the analysis. Environmental CPF concentrations in water (N=40) ranged 0.0118-2.857 µg/L, mean-SD 0.443±0.705 µg/L, median (25-75% quartiles) 0.171 (0.0761-0.432) µg/L. Sediment contained 2.90-242.5 µg/Kg, and CPF transfer to water was estimated in 0.0125-0.0197% considering physical-chemical properties of the samples. Exceedence probability analysis showed that water criteria were exceeded 75% (Argentina) to 87% (Canada) of cases. Risk assessment P5 endpoints were 0.0141 and 0.00050 µg/L CPF for acute and chronic effects in aquatic life respectively. These no conservative limits were respectively exceeded by 62% and 96% of cases in Tres Arroyos-Claromecó basin. Despite the relatively low CPF concentrations found in the basin, the probabilistic analysis suggests that a significant number of aquatic species may be seriously and unacceptably affected. The probability of exceeding water quality criteria for CPF concentrations in the region is also very high. If aquatic life protection criteria are compared with the probability risk curves, they should be established in 0.0005 µg/L considering P5-CPF chronic effects.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 42- HIGH THROUGHPUT (RNA-SEQ) SCREENING FOR BIOMARKERS OF AZINPHOS METHYL (AZM) EXPOSURE IN *Rhinella arenarum*

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Introduction: Azinphos methyl (AZM) is the main organophosphate pesticide applied in Río Negro-Neuquén valley in Argentina. In this sense, it is necessary to control the levels of residues and environmental impact of pesticides. Traditional ecotoxicological biomarkers like reduced glutathione (GSH) levels and the activities of Glutathione S transferase (GST) and Catalase (CAT) are commonly used for biomonitoring. However, they may fail for sensitivity and unspecifically respond to oxidative stress. Native species is a preferred resource for its ecological significance. In this sense, we have evaluated pesticide effects on common toad *R. arenarum* larvae. In this work, we screen for molecular biomarkers using RNA-Seq technology and compare the response with traditional biomarkers, in order to develop new specific and sensitive biomarkers. **Objective:** to develop new early, specific, sensitive biomarkers to AZM exposure. **Materials and Methods:** *R. arenarum* embryos were obtained by *in vitro* fertilization. Larvae (complete operculum (CO) + 11 days) were exposed to sublethal concentration of AZM (0.5 mg/L) [96h-LC50 10 mg/L)]. Samples were taken at 6h and 24h to evaluate GSH levels and the activities of GST and CAT. At the same time, samples were collected for RNA purification and massive sequencing. **Results and discussion:** We could detect changes only in GSH content but not in GST or CAT activities at 6h and 24h exposures to AZM. Regarding to the transcriptomic profile, we could determine up- and down-regulated genes compared with control for both 6h and 24h of exposure. After gene enrichment analysis, over representation of detoxification and oxidative stress pathways was found. Moreover, for Gene Ontology classification there were hits for Biological Process category such as cell differentiation, developmental growth, catabolic and hormone metabolic process, cell proliferation and cell cycle. **Conclusion:** here we show that transcriptomic analysis is a good tool to pick up gene candidates to be used as biomarkers. After gene expression analysis, we could detect changes in the level of several genes, even at early time of exposition (6h), while classical biomarkers did not show modification compared with control. Thus, after verification, some genes could be selected to develop more specific and sensitive biomarkers for biomonitoring pesticide impact.

EN 43- MONITORING CYTOTOXIC AND GENOTOXIC POTENTIAL IN WATER OF SINOS RIVER BASIN, SOUTHERN OF BRAZIL

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Introduction: *The environmental monitoring using biomarkers allows evaluate the environment contamination's degree through a bioassay association. Sinos River Basin (SRB) is an important source of drinkable for the state of Rio Grande do Sul, Brazil, however, this basin has suffered a series of recurring impacts over the years, which may reflect in the water quality and cause toxic effects.* ***Objective:*** *Monitor the cytotoxic and genotoxic potential in HEp-2 cells in water from seven points in the water the Sinos River Basin (SRB).* ***Materials and methods:*** *Water samples were collected in the three main rivers of SRB located in the cities of Santo Antônio da Patrulha, Rolante, Três Coroas, Taquara, Parobé, Campo e Esteio at nine different times during March 2012 and May 2013. After microfiltration the samples were used as a diluting solution of the culture medium for HEp-2 cells. The cytotoxicity was measured by the MTT and NR assay for which the cells were exposed to different concentrations of water for 24h and genotoxicity of the raw water (100%) was evaluated by the comet assay after exposure of 6 and 24 h. Results and discussion: Twenty samples from a total of 60 showed cytotoxic effect for the MTT assay and 30 for the NR. The point with the most cytotoxic samples was Esteio to MTT and Rolante to the NR. The months of March and May 2013 were the periods with the highest number of samples with cytotoxic effect. In a few samples was observed concentration-dependent cytotoxic effect (MTT for two samples and eight for NR). The dendrogram generated from the average of MTT and NR tests resulted in three groups with distinct similarities. The comet assay showed the presence of genotoxic substances in the water of the seven monitored points. Temporal and spatial variation was observed in genotoxicity results: all water samples of December 2012 caused DNA damage; Taquara and Parobe points showed the highest number of genotoxic samples. Considering the results obtained from MTT assays, VN and comet was possible through comparative analysis found that after 24 h of exposure, 6.6% of the samples were cytotoxic and genotoxic effects for the MTT assay and 18.3% for the VN test. It was also noted that some samples showed genotoxic effects after exposure, but significant effects of increased mitochondrial and lysosomal function.* ***Conclusion:*** *The results indicate that all the water stretch SRB has the potential to cause contamination with harmful effects on humans and aquatic biota. The assay with the HEp-2 cell line can be an additional tool for monitoring environment and contribute to the assessment of water quality.*

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 44- CULTURE OF GILL CELLS FROM *PROCHILODUS LINEATUS* TO USE IN ENVIRONMENTAL TOXICOLOGY

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Gill cells have been used in the xenobiotics toxicity evaluation in aquatic environments. The fish species *Prochilodus lineatus* (grumatã) has a wide distribution in the Sinos River basin (SRb) and its breeding in fish farms is usual. This study aimed to standardize a primary gill cell culture of grumatã for use in the cytotoxic evaluation of water samples from SRb. Fish of 10-20 cm were acclimated in dechlorinated water for 10 days with neomycin addition in the last 24h. After anesthesia in ice, fishes were decapitated and gills were harvested and washed (6 x 10 min) with saline solution containing antibiotics and antifungals. Then, samples were incubated twice in trypsin 1:250 for 10 min in 30ºC; cell homogenate obtained was filtrated in nylon mesh of 100 µm and centrifuged (400x*g*, 10 min). The cells obtained were maintained in Leibovitz medium supplemented with 15% of fetal bovine serum and antibiotics (Lbv-FBS/A) at 28ºC and closed system; 50% of the cells were adhered after 20h. The cultures were washed daily with saline solution and maintained in Lbv-FBS/A for until 14 days. Cell proliferation was observed only in the first 48h of culture, occurring cell hypertrophy after 7 days. During the first days of culture cells were stellate and/or fusiform; spherical nuclei with euchromatin and evident nucleolus were noted and a granulation was scattered in the cytoplasm and also on the extensions. After 14 days and staining with hematoxylin/eosin, we observed small groups of 10 or more cells characterized by an amoeboid aspect (58 µm), which the most peripheral ones presented several cytoplasmic extensions; another group were composed by large cells with stellate aspect (175 µm) and stress fibers. After isolation, 4.0 x10^7 cells were plated in a 24 well plate and maintained in same conditions described for one day. Then, the cells were exposed to the test mediums prepared with water samples from four points of water lands areas of SRb located at Rolante, Campo Bom, Novo Hamburgo and São Leopoldo municipalities. At the end of the exposure period (24 hours), cytotoxicity was determined by Neutral Red incorporation assay (NR). Cells maintained only with standard medium were used as negative control and cultures exposed to 1% hydrogen peroxide for one hour were the positive control. The results showed that primary cultures are responsive to the cytotoxicity assay, since the decrease of 80% was observed in lysosomal viability at the positive control. About the water samples, just Campo Bom was different from the negative control, with an increase of 85% in viability, indicating proliferative effect. Thus, we can say that we have success in isolating *Prochilodus lineatus* gills cells and that this primary cultures can be applied in environmental toxicology.

Financial support: CNPq, CAPES, Feevale

EN 45- 3M'S GLOBAL LCM PROCESS – MOVING TOWARDS SAFER ALTERNATIVES

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Introduction: 3M's global LCM (Life Cycle Management) process is used to identify opportunities associated with EHS&R (Environmental, Health, Safety and Regulatory) performance, and to characterise and manage EHS risks and regulatory compliance throughout a product's life cycle (raw material acquisition, development, manufacture, uses, and disposal). LCM is qualitative in evaluation and applies globally to all 3M products and internal transfers regardless of their source. **Goal:** The goal of LCM is to identify and manage EHS&R opportunities during the development of new products and sale of existing products. **Methods:** The LCM evaluations are done through the ELMS (Electronic Life Cycle Management System) database for individual or groups of similar products. This system is a centralized database for the storage, routing and retrieval of product EHS information. Each LCM document contains enough information regarding product (composition, manufacturing process, application) to conduct the risk assessments, such as, toxicological, environmental, industrial hygiene and others by SMEs (Subject Matter Experts). The toxicological and environmental assessments cover all the hazards, risks, precautions and disposal considerations involved in the manufacturing process and product use. **Results e Discussion:** 3M Brazil performed 455 LCM assessments by SMEs from January 2014 to June 2015. Once identified, the EHS&R issues were addressed in order to develop products that meet local and international regulations in addition to 3M's Policies and Standards. **Conclusion**: The LCM process and ELMS database provides a very efficient system that provides a single location for data entry of product information that allows the SMEs to evaluate a product's impact during its life cycle, adding EHS value and enabling 3M to launch safer products.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 46- ECOTOXICOLOGICAL RISK ASSESSMENT OF THE "ACID BLACK 210" DYE

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Introduction: Billions of dollars are involved annually in the production of dyes and pigments, but many studies have shown that these compounds can affect the aquatic life of the rivers near the industries or pose risks for the human consumption of these waters. The "Acid Black 210" dye is one of the most used dyes by the leather industry, it contains three azo groups in its chemical structure and has been quoted as a non-regulated dye with toxicological concern, since it could generate carcinogenic aromatic amines[1]. **Objective:** To do the ecotoxicological risk assessment of the "Acid Black 210" dye throught testing its mutagenicity in vitro and in vivo with the zebrafish embryo acute toxicity and the *Daphnia similis* test. **Material and Methods:** The mutagenicity studies were performed based on Maron and Ames method (1983), following the protocol described by Mortelmans and Zeiger (2000). The zebrafish embryo acute toxicity test was based on the procedures and endpoints described by the OECD guideline #286. The *Daphnia similis* test was performed according to the ABNT NBR12713:2009 descriptions, and the guidelines described in the OECD #202. All the tests used concentrations of the dye between 0.1 and 5,000 µg / mL. **Results and Discussion:** The zebrafish embryo acute toxicity test was negative for all the endpoints and tested concentrations (up to 100 µg / mL). Negative results were also obtained for the mutagenicity test with *Salmonella typhimurium* TA100 strain until the concentration of 5,000 µg / ml of the "Acid Black 210" dye. The same test with the *S. typhimurium* TA98 strain resulted in mutagenicity above 1,000 µg / mL, but with low mutagenic potency (0.0167 revertants / µg). The EC_{50} value for 48 hours in the *D. similis* test was 2,993.73 µg / mL. It is important to consider that according to the OECD #202, only EC_{50} values smaller than 100 µg / mL are relevant for classifying chemical compounds as with toxic concern. **Conclusion:** Due to the low concentrations of the "Acid Black 210" dye found in tannery effluents, and the high concentrations where any toxic activity is occasionally described, we concluded that this dye is safe from the ecotoxicological point of view in the light of the current knowledge. The toxicity observed in tannery effluents (data not shown) may be related to the presence of chemical components other than the dye used in this study.

References:

1. B. J. Brüschweiler et al. *Regul. Toxicol. Pharmacol.* 69:263, 2014.

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EN 47- THE EVALUATION OF CAFFEINE AND FLUOXETINE TOXICITIES FROM A GROWING CULTURE OF *Microcystis aeruginosa*

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Introduction: Environmental contamination comes from the improper disposal of sewage which leads to problems as eutrophication of water body. This process comes from the aplenty proliferation of micro and macro aquatic organisms compromising the quality of water. One of the problems is the production of cianotoxins by species like *Microcystis aeruginosa*. The microcystin is a hepatotoxic toxin that can cause danger to human health if present in drinking water. Some nutrients (N: P: K) and sewage have contaminants in their composition such as drugs. The effects of these compounds on cyanobacteria is not well known and some researchers have reported the cyanobacterial growth in presence of antibiotics (PLEITER et al, 2013; GRINTEN et al., 2010), antilipidemic (ROSAL et al., 2010), analgesics and anti-inflammatory (LAWRENCE et al., 2012). **Objective:** To evaluate the growth of *M. aeruginosa* with caffeine (CAF) and fluoxetine (FLX). **Materials and Methods:** *M. aeruginosa* inoculum was prepared from the LTPNA 08 strains in midst ASM-1 and used after 7 days of incubation at 20 ± 2 ° C and maintained on 12 hours light–dark cycle. Five concentrations, in triplicate, were evaluated for each compound (1000, 500, 100, 50 and 10 µg/L) and also prepared six concentrations as a positive control and one as a negative control. Each concentration was prepared by keeping a fixed amount of inoculum (1.68 x 104 cells/ mL). Samples of the growth of *M. aeruginosa* cultures grown under incubation (see above) were taken every 3 days for 18 days and analyzed by spectrophotometry method, in 1.5 ml cuvette (1 cm optical path length) at 750 nm. **Results and discussion:** The concentration of 10 µg/L of CAF appears to stimulating the growth in comparing to the control, for concentrations of 1000, 500, 100 and 50 µg/L. There was no significant inhibition in 9th day of growth (half of the experiment time), equal to 60.1%; 60.3%; 39.6% and 49.3% respectively. nor FLX the growth was considering equal to control in concentration of 10 µg/L, in 9th days the growth was inhibited around 50% for other concentrations. The value of EC 50 in 18th was obtained by plotting log (concentration) versus the inhibition percentage, equal to 34.49 µg/L for FLX and 31.08 µg/L for CAF. **Conclusion:** CAF appears to be toxic for *M. aeruginosa* in concentration above 50 µg/L whereas in concentration of 10 µg/L, CAF promotes the growth. Concentrations lower than 10 µg/L are being tested to investigate the environmental relevance in these datas. For FLX only the concentration of 10 µg/L was not toxic, which suggests that under environmental conditions (ng/L), this compound would not be toxic to this sort of cyanobacterium.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 50- MOTOR IMPAIRMENTS FROM METHYLMERCURY EXPOSITION AND ETHANOL IN FEMALE RATS FROM ADOLESCENCE TO ADULTHOOD

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Introduction: Ethanol (EtOH) is the most used drug of abuse by the young population, which consume increased especially among women. Ethanol intoxication in a binge drinking pattern is considered neurotoxic due to its capacity to promote nervous tissue damage, mainly during development. Another neurotoxicant substance is methylmercury (MeHg). It is an organic metal found in the ecosystem and a food contaminant via oral route during fish consumption. Thus, the association of these drugs may alter many areas in the brain especially the motor area. **Objective:** Analyze motor damage caused by the exposure to methylmercury and ethanol in female rats from adolescence to adulthood. **Material and method:** Ethical committee (BIO 209-14) approved this project. We used 40 Wistar female rats, divided in four groups (n= 10 rats/group): control, MeHg, EtOH and MeHg+EtOH. The groups were intoxicated orally using gavage method with MeHg (0.04 mg/ kg/ day during 35 days) and/or EtOH (3g/ kg/day during three consecutive days, once a week during 35 days of treatment). After the treatment period, the animals were submitted to behavior tests: open field test (OFT) and forced swimming test (FST). The statistical analysis was made by ANOVA one way and turkey test. **Results and discussion:** The open field test showed decreased travelled distance in EtOH group ($p<0.001$; $q=6.307$), MeHg ($p<0.05$; $q=4.125$) and MeHg+EtOH ($p<0.001$; $q=6.734$) when compared to the control group. The second parameter assayed on OFT was frequency of rearing, which was decreased in the EtOH group, MeHg and MeHg+EtOH ($p<0.001$; $q=8.175$; $q=10.90$; $q= 9.568$, respectively). On the FST was observed the frequency of climbing like a motor parameter, that was decreased only on MeHg+EtOH group compared to control ($p<0.05$; $q=4.362$), and compared to EtOH ($p<0.05$; $q=4.429$). The obtained results suggest that the MeHg and EtOH intoxication cause motor damage which compromise the animal locomotion. **Conclusion:** Our results demonstrated that motor alterations were caused by isolated exposure to EtOH and MeHg, and the association of these toxic compounds showed worst values on the behavioral tests.

EN 51- EVALUATION OF GLYPHOSATE TOXICITY ON MALES OF ZEBRAFISH *Danio rerio*

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Introduction: Several studies addressed the effects of glyphosate-based herbicides in a variety of non-target organisms, such as fish. In fact, glyphosate is a broad-spectrum organophosphate that may reaches the aquatic community, and consequently may affect non-target organisms, promoting a endocrine disruption. **Objective**: The aim of this study was to evaluate the reproductive toxicity of glyphosate on males of zebrafish *Danio rerio*, focusing on the somatic and germ cell responses. **Methods**: Males were acutely exposed to 65 µg/L of glyphosate [N-(phosphonomethyl)glycine] for 48 h, 96 h and 144 h. Additionally, chronic exposure was performed for 15 days. Non-exposed males were used as controls. Genotoxic test, to identify micronuclei in blood cells, was performed in order to recognize the glyphosate exposure effectivity. Then, testicles were dissected and submitted to ultastructural analyses and to cytotoxic assays by immunohistochemistry and flow cytometry to investigate the expression of Hsp70 and also, of anti- and pro-apoptotic proteins in the somatic and germ cells. **Results**: Our results showed a significant increase of the micronuclei only in males exposed to glyphosate for 15 days. In this same time, important changes on interactions between Sertoli and germ cells and loss of cytoplasmic bridges of the germ cells were recognized by transmission electron microscopy. However, after 144 h of exposure, a significant increase of the inducible Hsp70 was detected, which was not recognized in males exposed for 15 days. Regarding the proteins involved in induction of apoptosis, FASL showed a significant increase in males exposed for 15 days. But, no changes were observed on expression of Bcl2, Bak and active caspase-3. **Conclusions**: These results showed the cellular impairments induced by glyphosate and contribute to the understanding the glyphosate toxicity in non-target organisms. The noxious effects of glyphosate demonstrated here, point to a serious impact of this herbicide on male reproduction and must be taken into account.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 48- EFFECTS OF MEHG ON THE CELL DIFFERENTIATION IN THE MIDBRAIN CELL LAYERS

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Background: Methylmercury (MeHg) is an important neurotoxic agent and studies have shown high susceptibility of the CNS during development. The toxicological knowledge acquired on sublethal doses have great importance and can simulate the most common exposure forms. The mercury toxicity levels are dependent based on the compound type, also with the time of dose exposure and the lowest levels are the main source of contamination. **Aim**: The aim of this study was to analyze the effects of MeHg toxicity during cell differentiation and midbrain cell layers formation. **Methods**: A single dose of 0.1µgMeHg/50µL saline was applied in the egg at the third day of incubation (E3) and analyzed after one-week post treatment (E10). Control embryos received 50µL saline. The eggs remains on the incubator at 37.5°C and 65% RH. Embryos were fixed in 4% formaldehyde, dehydrated, embedded in paraffin to confirm the heavy metal deposition was used autometallography (AMG) method and for structural analysis of neural cell layers, sectioning at 6 µm and stained with hematoxilin-eosin. The expression of the cell differentiation-related proteins was evaluated by immunohistochemistry, using antibodies anti-β-tubulin III and Neu-N and anti-GFAP, as neural and glial markers, respectively. Unfixed midbrain was prepared for quantification by flow cytometry using the same cited antibodies. **Results**: Structural changes were not observed in the midbrain layers: ephendymal, mantle and marginal on the treated group embryos. It was identified that the number of cells labeled for β-tubulin III the group treated to MeHg is not significantly different to the control group. On the other hand a significant decrease in post-mitotic neurons (2373 ± 651.2; 768.2 ± 242.6, $p < 0.05$). For the glial lineage significant differences were not observed among the groups. **Conclusions**: The dose of MeHg used in this study did not cause changes in the structure of the cell layers of the midbrain, but changes at the molecular level were detected. Variations over time may disturb the equilibrium in the proliferation and differentiation of the neuronal lineage leading to the numerical change on these cells, however, without modifying the morphological development of mesencephalic layers, proving the importance of a preserved structurally system.

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EN 49- BEHAVIORAL CHANGES AND OXIDATIVE STRESS MECHANISM OF SUBCHRONIC INTOXICATION WITH METHYLMERCURY

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Introduction: Methylmercury (MeHg) is an organic metal, found in the environment, contaminating fish and marine life. According to Food and Agriculture Organization, the concentration of MeHg considered acceptable in the fishes range from 0.5 to 1mg/kg. However, low doses may be detrimental, and the population may be chronically exposed to low doses of MeHg by feeding. Studies indicate the involvement of oxidative stress as one of the mechanisms responsible for the toxic actions of MeHg, even at low doses. **Objective:** In the present study, we investigated whether subchronic MeHg exposure may induce neurobehavioral impairments. We also addressed whether oxidative stress may underlie these effects at a dose of the toxicant, which mimics the ingestion of food contaminated. **Material and methods:** Ethical committee (BIO 209-14) approved this project. Fourty-days-old female Wistar rats (n= 10 rats/ group) were administered with MeHg (40 mg/Kg/day) by oral route during 35 days. Control group received distilled water. The behavioral assays included open-field (OF), elevated Plus-maze (EPM), splash test (ST) and forced swimming tests (FS). The oxidative stress levels were measured in rat blood samples after behavioral assays and Trolox equivalent antioxidant capacity (TEAC), malonaldehyde (MDA), nitrite and nitrate (NOx), catalase activity (CAT), superoxide dismutase activity (SOD) and glutathione (GSH) levels were measured in vivo. Statistical analysis were performed by Student-t test for behavioral assays and one-way ANOVA, Tukey post-hoc for oxidative stress levels. **Results and Discussion:** Subchronic MeHg exposure decreased locomotor activity ($p< 0.05$; $t=2.297$), central locomotion ($p<0.03$; $t=2.902$), and grooming time ($p<0.03$; $t=3.785$) in the OF and ST tests, respectively. It also increased the immobility time in the FST ($p<0.03$; $t=5.059$). All behavioral results suggest that sub chronic MeHg at safe doses induces locomotion alteration, anxiety and depressant effects. In the oxidative stress assays, MeHg exposure reduced NOx ($p<0.001$; $q=15.76$) and increased CAT ($p<0.001$; $q=10.15$) and GSH ($p<0.03$; $q=5.689$), but did not alter TEAC, MDA and SOD levels in response to behavioral stress induced in rats. These data highlight that oxidative stress appears to be involved in these behavioral effects. MeHg generates free radicals, as H2O2, which requires higher CAT activity. The consequent generation of superoxide anion requires GSH action as well as the MeHg seems to be detoxified by it, since SOD was not induced. **Conclusion:** These results were indicative for the first time that subchronic MeHg exposure at safe doses induced locomotor alteration, anxiogenic, and depressant effects in female rats. Furthermore, the mechanism underlying these behavioral disorders may be related to the oxidative stress, even at MDA normal levels.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 52- MUTAGENIC AND ECOTOXICOLOGICAL ASSESSMENT OF THE COMMERCIAL TEXTILE DYE DISPERSE RED 73

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Introduction: Disperse dyes are organic substances which are insoluble in water and have low molecular weight, widely used in the dyeing of textiles products, toys, papers and other products. These compounds may reduce the survival of aquatic organisms, cause malformation, change gene expression and/or change biochemical parameters. Despite of that, they can induce contact dermatitis and allergies in humans. **Objective:** The objective of this work was to evaluate the mutagenicity and ecotoxicity of the Disperse Red 73 dye. **Material and Methods:** The mutagenicity of this compound was evaluated using assays with *Salmonella typhimurium* according to Maron and Ames (1983) and Mortelmans and Zeiger (2000), and the micronucleus assay was performed using HepG2 cells according to Natarajan e Darroudi (1991) with few modifications. The ecotoxicological evaluation was carried out according to ABNT NBR12713:2009 guideline employing the acute toxicity test with *Daphnia similis*. **Results and discussion:** The dye induced moderate mutagenicity without exogenous activation and low mutagenicity in the presence of exogenous activaction. The mechanisms of this mutagenicity are frameshift and base pairs substitution. The metabolic activation reduced the mutagenicity of the dye, possibly because the products generated after the action of cytochrome P450 isoforms were less reactive with DNA, which does not indicate the complete detoxification of the compound, since only the mutagenicity endpoint was evaluated. However, the dye did not induce micronucleus formation in HepG2 cells in different treatments compared to the negative control. The Disperse Red 73 dye was classified as extremely toxic to the planktonic microcrustacean *Daphnia similis*. **Conclusion:** The Disperse Red 73 dye is capable of causing adverse effects at low concentrations, and this is of great concern because these low concentrations could easily be found in the environmental do to the estimated release of 1.2 tons per day of dyes in the aquatic environment related to the low fixing rates of the dyes to fibers. Additionally, it is important to consider that the consumer's market requirements for color endurance in the colored fibers even after processes as sweating, washing and exposure to sunlight, result in the development of more stable chemical structures of dyes, increasing the half-life time of these compounds in the environment, which could reach almost 50 years.

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EN 53- COMMERCIAL REACTIVE BLUE 4 DYE INDUCES HORMESIS EFFECT IN *CERIODAPHNIA DUBIA*

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Introduction: Reactive dyes are used in the cotton, wool and polyamide fibers dyeing processes. Although they have a growing economic market, toxicologists are concerned about the difficulties in the treatment of their effluents, especially because they have poorly absorbed by biomass, and they are not degradable in the aerobic conditions used in the conventional wastewater treatment. Additionally, they have low fixation rates on fibers, which results in large losses to the environment during the dyeing bath. Therefore, the reactive dyes can be easily found in the aquatic ecosystem. **Objective:** The objective of this work was to evaluate the eco and genotoxicity of the Reactive Blue 4 dye using the acute toxicity tests with *Daphnia similis* and *Vibrio fischeri*, the chronic toxicity test with *Ceriodaphnia dubia*, the genotoxicity (Comet assay) test with human dermal fibloblasts, and the mutagenicity test with *Salmonella typhimurium*. **Material and Methods:** The ecotoxicological assays with *Daphnia similis, Vibrio fischeri* and *Ceriodaphnia dubia* were performed according to ABNT NBR12713:2009, ABNT NBR15411-3:2012 and ABNT NBR 13373:2010 guidelines, respectively. The Comet assay was carried out according to Tice et al. (2000) and the *Salmonella typhimurium* mutagenicity assay was carried out according to Maron and Ames (1983) and Mortelmans and Zeiger (2000). **Results and Discussion:** The Reactive Blue 4 dye was moderately toxic to *Daphnia similis* and reduced *Vibrio fischeri* luminescence in high concentrations in the short-term assays. The dye also induced hormesis effect in the experiments with *C. dubia*, since the reproduction of this water flea was stimulated at low concentrations of the dye, followed by a decrease of it at higher concentrations. The daphnids exposed to stressors may increase the size of the offspring, but the reproduction may be delayed and the eggs had their size reduced. The change in the distribution of biomass in number and size of the daphnids' eggs is an relevant ecological adaptation in variant environmental conditions, as an attempt to keep the population even in adverse conditions. The dye induced low mutagenicity by substitution of base pairs in the presence of exogenous metabolites, probably by the formation of compounds more reactive with DNA, and the dye did not cause genotoxicity in human dermal fibroblasts. **Conclusions:** The Reactive Blue 4 dye is a relevant environmental contaminant, since the results show that this dye can cause adverse effects on aquatic organisms even at low concentrations, and the continued discharge of this compound in water bodies is worrying.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 54- RELATION BETWEEN THE GENOTOXICITY OF AIR PARTICULATE MATTER AND THEIR CHEMICAL COMPOSITION

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Many organic and inorganic compounds, identified as hazardous contaminants by their mutagenic capacity are present in the air particulate matter (PM) in urban environments. Epidemiological studies suggest that continuous exposure to particulate matter is associated with increased mortality and morbidity, and also with long-term effects such as lung cancer. Usually, in the evaluation of the air pollution, the concentrations of PM and some specific pollutants are determined. However, this method can only assess the current condition and does not provide any data on the impact on exposed organisms. Therefore, in addition to analytical procedures, the application of bioassays is necessary to identify the genotoxic effects of complex mixtures such as PM and can define preventive actions to control the environmental quality. In order to establish the relationship between the concentrations of some compounds in samples of PM and their potential genotoxic effect, we extracted the organic and inorganic water soluble fraction of twelve filter samples (TSP and PM10) collected in the state of São Paulo and determinate fifteen soluble metals (ICP) and the sixteen EPA´s priority polycyclic aromatic hydrocarbons (HPLC). The mutagenic activity was assessed by the Salmonella/microsome assay (Ames Test) using *Salmonella enterica* serovar Typhimurium strains TA98 and TA100, with and without *in vitro* metabolic activity. Additionally, we calculated the Benzo (a)pyrene mutagenicity equivalent factor (MEQ BaP) as a way to evaluate the potential risk. The twenty-four organic extracts showed mutagenic activity and only three inorganic extracts were negative under all test conditions. Our results show that the presence of mutagenic compounds in the samples do not explain all the biological effect observed, as there was a weak correlation between the concentrations of the compounds tested and the mutagenic potency. Our data confirm that the evaluation of the potential risk of exposure to PM based on chemical analysis may be underestimated. Not only it is not possible to determine all chemical compounds within any environmental complex sample, a variety of interactions between the compounds can interfere in their mode of action, confirming the importance of biomonitoring studies to ensure the safety of exposed populations.

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EM 55- RISK MANAGEMENT AT MANGUINHOS, RIO DE JANEIRO CITY (RJ) – BRAZIL

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Introduction: Manguinhos is located in the watershed of the Cunha Canal, formed by Jacaré and Faria-Timbó rivers, on the eastside of the Rio de Janeiro city, in about an area of approximately 63.6 square kilometers that includes 13 slums, the campus of Fiocruz, the old Orphanage of Cristo Redentor areas and Manguinhos oil refinery, with an estimated population of 36 000 people. Historically, Manguinhos surrounding has many environmental damages from industrial sites in decline. The existence of a population dense settlement without proper sanitation infrastructure contributes to increase health problems. Several studies have described harmful impacts on the environment and human health from oil refineries pollution in Brazil, however the review does not describe any results about Manguinhos oil refinery impacts. The chemicals from the oil refining waste are toxic and impact public health and the environment, mainly the Polycyclic Aromatic Hydrocarbons (PAHs). The Brazilian Ministry of Health adopts (ATSDR) **Agency for Toxic Substances and Disease Registry** tool that considers community concerns about their health and related indicators. It should involves public health potential impacts communication to local community and recommend protection actions. Because of all this facts, it is important to explore Manguinhos environmental contamination problem. **Objective**: The aim of this study is to assess PAHs soil contamination in order to define indicators to quantify human and environmental health risks. **Materials and Methods**: The surface soil sampling plan was distributed in the area with 72 points with 150meters between each other divided in 4 series, which were tested for PAHs contamination by GC/MS. **Results**: The range of PAH trace detection was defined from zero to 24,4mg/kg, after the data was treated by the median value and it results on 72 results. These results were compared with recommended values from CETESB (órgão ambiental de São Paulo). The finding was that of the 72 sample points, 12 had smaller values than the reference values (0.2 mg/kg); 58 had values between Reference Values and Precaution Values (4,1mg/kg) and only one, value higher than Intervention Value (8,1mg/kg). **Conclusion**: These results indicate that it is necessary hazardous waste disposal management and improved control because the studied site is in the surrounding of the Manguinhos Refinery. The results also suggest that the refinery soil where contains higher PAHs levels than those obtained for the Manguinhos neighborhood soil.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 56- INHALATION OF FINE PARTICLES FROM MEXICO CITY INCREASES THE EXACERBATIONS IN A GUINEA PIG ASTHMA MODEL

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Introduction: Air pollution is a big problem around the world, mainly in mega cities, where contaminants increases day to day. In Mexico City, both industrial activity and their over 5.5 million of vehicles produce gases or particulate matter (PM) emissions. Particles can remain in the lung for a long time. Also, they are composed of different elements such as: metals, non-metals, metalloids, polycyclic aromatic hydrocarbons, volatile organic compounds, oxides, glucans, endotoxins, pollen, and viruses. Several environmental factors may contribute to allergic disease such as, asthma development. Some reports have mentioned that, ozone exposure increases asthma attacks after inhalation, but exposure to PM is controversial. Other studies have instilled PM in asthmatic rat or mouse. In both cases it has been reported increases of asthma exacerbations. **Objective:** Our aim was to determine whether inhalation to fine particles from Mexico City can increase the asthmatic exacerbations in a guinea pig model, since it faithfully reproduces to human airway anatomy, hyperreactivity and inflammatory response. **Methods:** Animals were sensitized to ovalbumin (OVA) plus $AlOH_3$ (i.p. or s.c.) Using an Aerosol Concentrator System in the North of Mexico City, (CINVESTAV-Zacatenco), animals were exposed to filtered air (FA) or PM2.5 (daily 5h/3days). On the 21th day we evaluated lung function by plethysmography to obtain an airway resistance index after OVA challenge. Animals were euthanized, and bronchoalveolar lavage was performed to determine the percentage different cellular elements. Lung samples were recovered for histology and PAS-stained. **Results and Discussion:** Our results showed that airways resistance index after OVA challenge increased in asthmatics exposed to filtered air (FA) or fine particles (FP). Asthmatics exposed to FP responded faster than asthmatics exposed to FA. Asthmatics exposure to FA increased mainly eosinophils whereas asthmatics exposed to FP showed neutrophilia. Also, mucous cells increased in exposure to FP than exposure to FA. **Conclusions:** Our results suggest that inhalation of fine particles from Mexico City in asthmatic model can produce obstruction increases and exacerbations. However, we could associate it to the neutrophilia as a forecast of fatal asthma.

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EN 57- INFLUENCE OF AGE AND LEVEL OF EXPOSURE ON ARSENIC METABOLISM IN A POPULATION CHRONICALLY EXPOSED TO ARSENIC THROUGH DRINKING WATER

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Arsenic exposure through drinking water is a public health problem in Argentina. It is estimated about two million people exposed. The influence of the age and the level of As exposure on arsenic (As) urinary metabolic profile was evaluated on chronically exposed inhabitants from rural and urban locations of the Chaco Pampean Plains in Argentina. Population was composed by 157 children up to 12 years old and 73 adolescents and adults. Urinary inorganic As (IAs) and methylated metabolites, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were measure for all participants by HPLC-HG-AAS. Urinary As (UAs) was set as indicator of As exposure, it was calculated as the sum of all the As species (IAs+MMA+DMA) and expressed as µg of As per g of creatinine. Population was grouped according to the level of exposure into three levels: low (L), moderate (M) and high (H) exposure (UAs up to 100, between 101 and 500, and more than 500 µg/g creatinine, respectively). Wilcoxon Mann Whitney U test and Spearman correlation coefficient (*Rs*) were applied for statistical analysis. Statistically significant differences were observed in the urinary MMA (17 and 20 %, $p = 0.0011$) and in the urinary DMA (69 and 64 %, $p = 0.0126$) between children and adults, respectively. No difference in urinary IAs between the two groups was observed ($p > 0.1$). Same analysis was performed for children and adults at each level of exposure. Results showed statistical differences for the MMA (17.8 and 22.4 %, $p = 0.0001$) and the DMA (62.2 and 59.3, $p = 0.0002$) for children and adults, respectively, only for participants with high level of exposure (group H). For participants with medium and low levels of exposure, the differences were not statistically significant. A statistically significant correlation between age and % MMA (positive, $Rs = 0.15$, $p = 0.0195$) and % DMA (negative, $Rs = -0.18$, $p = 0.0073$) was observed when analyzing the whole population. No correlation was observed when the two groups, children and adults, were analyzed separately. However, it seems to be a slight increase in the % MMA with age, in adults. Results showed a difference on As urinary metabolic profile between children and adults. This difference could not be attributed to age, but to the fact to be children or adults. Also the difference was observed at high levels of exposure suggesting that, at moderate to low levels of exposure, adults methylation capacity was effective enough to metabolize As as well as children's. Moreover, S-adenosylhomocysteine (SAH) is a potent inhibitor of As3MT and children have lower levels of homocysteine (precursor of SAH) than adolescent and adults. This fact could add for the observed difference in methylation capacity at high levels of exposure. However, additional studies including homocysteine and/or SAH quantification are needed.

Sci Total Environ. 429:76, 2012
J Toxicol. 2012:595307, 2012
J Nutr. 133:2643, 2003

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

EN 58– IS NATURAL RADIOACTIVITY A PUBLIC HEALTH CONCERN TO BE TAKEN INTO ACCOUNT IN BRAZIL?

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Radionuclides of natural origin are normally present in different amounts in the environment. They are released from rocks and minerals. In the Brazilian semiarid region is settled down more than 22 million of inhabitants. The terrestrial crust in this region has one of the most important reservoirs of uranium worldwide. Nevertheless little is known about natural radioactivity as a human health concern. This work was aimed to assess human population exposure to natural radioactivity. A one year follow-up study was performed in 411 houses from three cities located in the Rio Grande do Norte State/ Brazil where indoor radon (Rn) concentration, through the passive emanometry method (BGO crystal), along with uranium (U), thoronium (Th) and potassium (K), by the gammamtric method, were measured. High levels of indoor Rn were observed (mean: 343.9 Bq/m^3; range: 4563.1 Bq/m^3). Mean concentration of U, Th and K was: 3.77ppm; 14.61ppm and 3.14% respectively. The total (Rn+U+Th+K) mean annual effective dose was 6.5 mSv/y with a range of 80.5 mSv/y. Considering a cut off radon level of 148 Bq/m^3 (EPA 2015), 67% of the houses presented a Rn level in hazardous concentrations for human exposure. The estimation of the annual effective dose showed that 30% of de population settled down in the area studied is classified as middle-very high exposure (UNSCEAR 2015). Natural radiation is the main source of radiation in the surrounding environment contributing to human exposure in approximately 90%. Radon is the second cause of Lung cancer worldwide. Because of the significant health hazards associated with radon, its concentrations are widely monitored throughout the world. Nevertheless information about the relevancy of natural radioactivity on public health on Brazil is very scarce yet. The results obtained demonstrated radioactivity levels as high as the most affected areas worldwide, and the need to consider natural radioactivity as a human healh concern to be taken into account.

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EN 59—TOTAL LEVELS OF MERCURY IN SEAFOOD CAPTURED IN ARATU BAY, BAHIA.

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Introduction: The human interference on environment has been increased dramatically in the last centuries, causing pollution and degradation of ecosystems. The most affected by this impact is the aquatic ecosystem, which are affected by industrial, mining and agricultural effluents. The Aratu Bay, an estuary of the Todos os Santos Bay, has a small extension which concentrates several industrial activities, among them textile, pharmaceutical, mechanical metal, naval, chemical and plastic companies, all of them with great potential to generate waste with heavy metals, including mercury (Hg). This element has biogeochemical cycle that involve a very toxic organic form, methyl-mercury, which has a great potential for bioaccumulation and biomagnification in the food chain. Thus, the biome of these waters may be bioaccumulating these metals in their tissues, and the population that eats these seafood are potentially exposed. **Objectives**: Evaluate the levels of total mercury in fish (shrimp, crab, oyster, miroró, sururu and pititinga) captured in the Aratu Bay in the community of Santa Luzia, district of Simões Filho, Bahia. **Materials and Methods**: For this assessment six species of fish and shell-fish were used. Samples were acquired from artisanal shellfish pickers bimonthly from November 2013 to September 2014. The samples were freeze-dried and an aliquot of 100 mg were subjected to hot acid digestion by 6 hours. The determination of Hg was performed by atomic absorption spectrometry with cold vapor generator. All samples were analyzed in triplicate. The accuracy was assessed using the recuperation method. Precision was evaluated in terms of relative standard deviation. The reference material used was IAEA 085 human hair. **Results and Discussion**: The Brazilian legislation establishes a maximum limit of 0.5 mg Hg/kg for non-predatory fish and 1.0 mg/kg for predator fish. The values obtained in all sets analyzed along the period were below the limit allowed by law, however, crab samples presented the highest average concentrations: 0.0796 ± 0.0014 µg/g; 0.1110 ± 0.0128 µg/g; 0.0894 ± 0.0110 µg/g in January, March and May 2014, respectively. The highest values observed in crab samples could be explained by the fact that this decapods filters seawater, and feeds on other animals corpses and thus could be biomagnifying mercury compounds in their tissues. **Conclusion**: The values observed show a low risk to the health of the population; however a very rich diet with such seafood could pose a dangerous exposure at long term, increasing the risk of deleterious health effects.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 60- SOMATOSENSORY PSYCHOPHYSICAL LOSSES IN MERCURY-EXPOSED OF RIVERSIDE COMMUNITIES OF THE TAPAJÓS RIVER BASIN, AMAZON, BRAZIL

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Introduction: It is generally feared that chronic exposure to mercury might be potentially dangerous to human health and the possible occurrence of neurological effects due to mercury toxicity is of great concern. Few studies evaluated the sensory function of mercury-exposed inhabitants of Amazonian riverside communities and they all have reported cognitive, motor, and sensory impairment associated to the mercury exposure. **Objective**: To evaluate the somatosensory system of methylmercury exposed inhabitants living in the communities of the Tapajós river basin by using psychophysical tests and to compare with measurements performed in inhabitants of the Tocantins river basin. **Methods**: We studied 108 subjects from Barreiras and São Luiz do Tapajós, two communities of the Tapajós river basin, State of Pará, Amazon, Brazil, aged 13-53 years old. Mercury analysis was performed in head hair samples weighting 0.1-0.2 g by using atomic absorption spectrometry. Three somatosensory psychophysical tests were performed: tactile sensation threshold, vibration sensation duration, and two-point discrimination. Semmes-Weinstein 20 monofilaments with different diameters were used to test the tactile sensation in the lower lip, right and left breasts, right and left index fingers, and right and left hallux. The threshold was the thinner monofilament perceived by the subject. Vibration sensation was investigated using a 128 Hz diapason applied to the sternum, right and left radial sides of the wrist, and right and left outer malleoli. Two trials were performed at each place. A stopwatch recorded the vibration sensation duration. The two-point discrimination test was performed using a two-point discriminator. **Results**: Head hair mercury concentration was significantly higher in mercury exposed inhabitants of Tapajós than in non-exposed inhabitants of Tocantins ($p < 0.01$). Tactile sensation threshold in mercury exposed subjects was higher than in non-exposed subjects at all body parts, except at the left chest. Vibration sensation duration was shorter in mercury exposed than in non-exposed subjects, at all locations except in the upper sternum. Two-point discrimination threshold was higher in mercury exposed than in non-exposed subjects at all body parts. Tactile sensation threshold, two-point discrimination, and vibration sensation duration at some body locations provided the highest proportion of impaired subjects (≥ 30%). There was a weak correlation between tactile sensation threshold and the mercury concentration in the head hair samples. **Conclusion**: Mercury exposed subjects had impaired somatosensory function compared with non-exposed control subjects. **Significance**: Long-term mercury exposure of riverside communities in the Tapajós river basin is the likely cause of psychophysical somatosensory losses observed in their population.

Acknowldgements: Financial support from CNPq/MCT Projeto Universal,2012

EN 61- THYROID AND IMMUNE FUNCTION IN THE PROLONGED EXPOSURE TO MERCURY IN BRAZILIAN AMAZON

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Introduction. There are evidences that mercury accumulates in the thyroid and can cause endocrine and immune disturbances. Riverside population in the Amazon River present long-time exposition to mercury originated from food and the neurological effects have been available, however, there are few studies about the specific disturbances of hormones and immunes of the thyroid. **Objective**: to evaluate if the long-time exposition to mercury is associated with alteration in thyroid hormones and change in anti-TPO antibody. **Methods**: The study included 79 people of riverside communities of the Tapajos River exposed to mercury for a long time. The participants were between 14 to 54 years old, both men and women. The measurements of hormones concentration in serum (TSH, T3 and T4 free) were analyzed by *ELISA* technique and titles of Anti-TPO, by immunoenzymatic method. Total Mercury, in samples of hair, was measured by spectrophotometry of atomic absorption by technique of hot vapor. **Results and discussion**: The frequency of high TSH was 5.3% with maximum value of 8.9µU/ml, considered within the standards for subclinical hypothyroidism, in absence of high T3 and T4-free. The frequency of levels of TSH below the reference limit indicating subclinical hypothyroidism was 2.3%. Titles of Anti-TPO were normal in all participants. There was no correlation of hormones TSH, T3 and T4-free, neither of immune in level of exposition to mercury. **Conclusion***:* the levels of long-time exposition to low concentration of mercury are not associated to the hormones and immunes of the thyroid, probably because other individual and environmental factors were influencing the thyroid answer.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 62- INTERVENTION OF A DRUG DISCARD PROGRAM IN THE PREVENTION OF POSSIBLE ENVIRONMENTAL IMPACTS: A PILOT STUDY

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Introduction: Improper drug discard can trigger serious environmental damage, because to be released into the environment can interfere with many living systems. The marketing influence, dispensation beyond the exact amount for the treatment and distribution of free samples, associated with absence of knowledge of the general population about the question, are the main factors contributing to incorrect drug discard and, consequently, for environmental contamination. **Objective:** Characterize discarded drugs by the population of Guanambi-BA through a drug discard program. **Materials and Methods:** Were included in this pilot study adults of both genders users of medications and living in Guanambi-BA who went to the fair collection of expired drugs held by the proposing institution. The volunteers were informed about the research procedures and answered a specific questionnaire that assessed aspects associated to drugs discard. The expired and unused products which were donated by the participants were measured and grouped according to ATC (Anatomical Therapeutic Chemical) classification. The SPSS v.20 software was used for data tabulation and carrying out of descriptive statistical analysis. The whole procedure was approved by the Research Ethics Committee. **Results and Discussion:** Were received altogether 146 drugs donations. The formulations were classified into 111 different types according to ATC classification, and the most common were the drugs that act on the gastrointestinal tract and metabolism (21.6%) and the cardiovascular system (18.9%). Eighty-seven point six percent of the drugs were expired and the similar represented 56.9% of total, followed by generic (21.5%), reference (16%), free sample (3.5%) and others (2.1%). The most present pharmaceutical form of the donated drugs was the tablet (62.3%) which, together with capsules and dragees, totaled 2383 units donated. Approximately 44% of respondents reported not knowing how should be dispose of expired drugs and 33% said that they discard expired medications in the garbage, which is corroborated by others studies. Thus, this immediate solution for the discard of waste medicines is among the main measures adopted by the population, predominating discard in the garbage, which can cause significant environmental damage. **Conclusion:** It can be concluded that the absence of information on correct drug discard still is great and many of the expired drugs of Guanambi city can go to undue place and therefore to contaminate the environment.

EN 63- BLOOD ANTIOXIDANT NUTRIENTS IN RIPARIAN VILLAGERS OF THE BRAZILIAN AMAZON: LINKAGES WITH WET/DRY SEASONS AND MODULATION BY SOCIODEMOGRAPHIC DETERMINANTS

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Introduction: Several studies have shown that sociodemographic and lifestyle features of the population such as sex, age, body mass index (BMI), smoking habits, alcohol consumption, the presence of pathologies, among other variables, may also affect blood levels of essential trace elements and vitamins. **Objectives:** The aim of this study was to relate blood antioxidant nutrients levels to wet/dry seasons and socio-demographic determinants in small-scale riparian communities of the Brazilian Amazon. **Material and Methods:** The studies were carried out during the rainy and the dry seasons, while investigating blood antioxidant nutrients levels copper (Cu), manganese (Mn), selenium (Se), zinc (Zn), carotenoids (β-carotene and lycopene) and vitamins (A and E) with respect to sociodemographic features of the population. Three agricultural communities were investigated, located in the Tapajós River region, Pará, in the Brazilian Amazon. Adult population comprised of 84 participants during the rainy season (RS), and 103 persons for the dry season (DS). Data were interpreted along with the Man-Whitney U and Kruskal-Wallis H tests. **Results, Discussion and Conclusion:** Cu, Se, Zn and lycopene were significantly lower in the rainy season as compared to the dry season ($p<0.05$). On the opposite, Plasma β-carotene and vitamin E were higher in the rainy season with respect to the dry season ($p<0.05$), while blood Mn and vitamin A were not influenced by seasonality. Se, Mn and β-carotene presented intercommunity variation ($p<0.05$), whereas age, BMI and years of education had no influence on the micronutrients evaluated ($p>0.05$). During dry season, blood Zn was higher in men than women ($p=0.04$), and smokers had higher Se than non-smokers ($p=0.02$) while villagers who both farmed and fished had higher Cu and lower β-carotene than participants with a single occupation ($p=0.04$). In both seasons, drinkers had lower β-carotene than non-drinkers ($p<0.0001$) while for vitamin E this only held true in the DS ($p=0.04$). In both seasons, vitamin A was higher in drinkers than non-drinkers ($p=0.03$). Drinkers' lycopene values were only lower than non-drinkers' during dry season while vitamin E levels were highest in natives of Pará state ($p<0.0001$ for both) as compared to persons born outside the state. In riparian communities of the Brazilian Amazon, blood antioxidant nutrients levels are influenced by seasonality, then modulated by community location, sex, smoking, alcohol consumption, occupation and origin. This study highlights for the first time carotenoids and vitamins blood levels for Tapajós River populations. It sets baseline values in two different seasons for populations characterised with a unique lifestyle and eating habits, which change considerably according to seasons.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 64- ACUTE TOXICITY OVER *Ceriodaphnia silvestrii AND Daphnia magna:* BIOASSAYS WITH WATER SAMPLES FROM A DAM UNDER THE INFLUENCE OF URANIUM MINE AND WITH MANGANESE

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Introduction: Treated effluents from uranium mine (UTM/INB) with acid mine drainage (AMD) can negatively impact adjacent receiving water bodies. AMD is relevant from the environmental point of view, mainly due to the large volume of effluents generated, which are known to adversely affect aquatic biota. Thus, the formation of this acid mixture, consisting of a large spectrum of chemical pollutants, creates a harsh environment that is harmful to aquatic organisms living around the mining area. Manganese is a toxic element, frequently overlooked in the assessing the toxicity of effluents, in which this metal can be present at toxic levels. Numerous studies have shown that one of the main problems of treated effluents, released by UTM/INB on the catchment basin of Ribeirão das Antas, is associated to high manganese values recorded in water samples. **Objective**: In this context, preliminary toxicity tests with manganese were conducted with bioindicators species *Ceriodaphnia silvestrii* and *Daphnia magna*. **Materials and Methods***:* to determine the EC_{50} values of Mn to cladocerans in toxicity laboratory bioassays. In addition, we compared these EC_{50} values with water samples Mn concentrations from the Antas Dam, which receives treated effluents from UTM/INB. **Results and Discussion**: In the present study preliminary results of acute toxicity for *C. silvestrii* indicated 100% of immobility at 9.0mg Mn/L and 0% of immobility at 3.0mg Mn/L. For *D. magna,* 30 mg Mn/L caused 0% immobility to organisms and 90mg Mn/L caused no effect. It was verified that Mn concentrations determined in environmental samples registered the highest value at 1.75mg Mn/L, indicating that they were below the lethal concentrations recorded for both species. **Conclusions**: Since manganese occurs in the composition of the effluent that contains other stable and radioactive elements, it is crucial to conduct complementary ecotoxicological tests, aiming at the assessment of possible synergistic and antagonistic effects of the chemical mixture that makes up the radioactive effluents that are treated and released at the Antas Dam. Such bioassays are underway in the Radioecology Laboratory at LAPOC/CNEN.

Acknowledgments. The authors would like to thank the analytical chemistry and radioecology technicians at the Poços de Caldas Laboratory/Brazilian Nuclear Energy Commission (LAPOC/CNEN) and Poços de Caldas Department of Electricity and Distribution (DMED-Poços de Caldas, MG) for financial support.

EN 65- IMPLEMENTATION OF BIOASSAYS FOR ECOTOXICOLOGICAL TESTS ON RADIOACTIVE EFFLUENTS, IN ACCORDANCE WITH THE METHODOLOGIES: ABNT, OECD AND ISO-6341

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Introduction: *Daphnia magna* and *Ceriodaphnia silvestrii* have been used in acute and chronic toxicity tests in many countries for a long time. Toxicity tests are often used as applicable tools to detect and assess the inherent capacity of the toxic agent to cause possible deleterious effects in bioindicators. Moreover, acute and chronic toxicity tests complement the physical, chemical and radiological data of the environment and contribute to a better evaluation and risk prediction in sediments, contaminated waters and radioactive effluents. Ecotoxicological tests are being conducted on water samples from the uranium mine (UTM/INB) and its drainage basins, in addition to tests with stable and radioactive metals of interest. **Objective**: Toxicity bioassays methodology implementation to *C. silvestrii* and *D. magna* in the Laboratory of Radioecology (LAPOC/CNEN). **Materials and Methods**: The methodology implementation of *C. silvestrii* culture was conducted according to standards ABNT NBR-13373 (2010) and, while for the exotic D. magna species, the ABNT NBR 12713 (2009) and international standards OECD (2004) and ISO-6341 (2012) were followed.
Results and Discussion: The results indicated that the use of synthetic water distilled (50%) and MilliQ-deionized (50 %) for preparation of reconstituted water, which was used in the cultures, demonstrated improvement at growth rate and organism reproduction. In this condition, there was reduction of egg-bearing female deaths, absence of males and an increase in the number of viable generations and neonates. Such culture conditions allowed the fulfillment of the standard requirements, or the continuous maintenance of the parthenogenetic reproduction, avoiding resistance eggs and overpopulation in the *D. magna* culture. For nourishment of *C. silvestrii* and *D. magna* cultures, the conditions of the algae *Raphidocelis subcapitata* culture were optimized: algal cell culture amidst CHU, temperature of 25° C and axenic conditions led to algal density values up to 3.98×10^7 cel/ml. The sensitivity tests conducted with standard reference substances NaCl (*C. silvestrii*) and $K_2Cr_2O_7$ (*D. magna*) presented values within the recommended sensitivity range: for *C. silvestrii* the CE50 48h was 1.4 g/L of NaCl while for *D. magna* the CE_{50} 48h values ranged from 1.05 and 1.29 mg/L of K_2Cr2O_7. **Conclusion**: Under the culture conditions that were optimized with the implementation of methodologies in the Laboratory of Radioecology (LAPOC/CNEN), both species are in accordance with the requirements in the Standards ABNT, OECD and ISO-6341, and thus appropriate for employment in acute and chronic ecotoxicological tests.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 66- DIFFERENT END-POINTS TO ASSESS EFFECTS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS EXPOSED TO PESTICIDE MIXTURES

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease with high female predominance, in reproductive years. It is characterized by a pronounced inflammation and production of a variety of autoantibodies. SLE pathogenesis is influenced by genes, hormones and environmental agents. **Objective:** To assess the possible effect of environmental pesticide mixtures in SLE patients. **Materials and Methods:** Oxidative DNA damage was measured using the comet assay modified by enzyme Endo III, for detection of oxidized bases (Endo Sites), and oxidative stress from the measurement of the activity of catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation (TBARS). Eighty-nine patients with diagnosis of SLE were included, 46% of them came from areas highly sprayed with pesticides and were compared with patients from urban areas with the same clinical and sociodemographic characteristics ($p \geq 0.155$). **Results and Discussion:** The analysis of rural patients showed that most of the population live within 500 meters from the crops. Despite being a community closely linked with agricultural activities, they showed little knowledge about which pesticides are applied in the field area and which are the protective measures to be used by rural workers. The presence of glyphosate, AMPA, atrazine, 2,4-D and chlorimuron ethyl were detected in samples of suspended particulate matter and rainwater of the studied region. In order to identify factors that could predict DNA damage and oxidative stress, a binary logistic regression model with independent variables was obtained: place of residence ($p = 0.007$) and smoking habit ($p = 0.186$) have 75% of positive predictive value and 56% negative predictive value, respectively. The Odd Ratio obtained indicate that lupus patients living in rural areas presented 3.52 times more oxidative DNA damage compared to those living in the city. In case patients have been a smoker the ratio decrease to 2.06 times. The prospects of applying new biomarkers to assess exposure and biological effects, such as DNA damage and oxidative stress in autoimmune diseases allow improving the ability to characterize individual risk. **Conclusions:** This research demonstrates the importance of using biomarkers of oxidative DNA damage in clinical monitoring of SLE patients, in order to establish appropriate treatment guidelines and the relationship between environmental pesticide exposure and increase in oxidative DNA damage in SLE patients.

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EN 67- ASSESSMENT OF GENETIC DAMAGE IN REPRODUCTIVE AGE WOMEN CAUSED BY ENVIRONMENTAL EXPOSURE TO PESTICIDES

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Introduction: Pesticide exposure and women's health information from developing countries is scarce and frequently not published in the international scientific literature. Forty-four percent of the total agricultural labor force in developing countries are female. Women may be occupationally- or environmentally exposed to pesticides; however, their perception of the risk is low. Many of the effects of pesticides in human health could be the same for males and females, but sex-related biological differences strongly support a distinct susceptibility to the toxic action of these chemicals. Besides, it should be considered that sometimes women are pregnant or accompanied by their children in an unhealthy environment. **Objective:** To evaluate the action of pesticides in women of reproductive age using biomarkers of exposure and genotoxicity. **Materials and Methods:** The variables used to describe pesticide exposure were butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), and to determine genotoxic effects were: comet assay in peripheral blood leukocytes (DI), their modification to determine oxidized pyrimidines using specific repair enzyme ENDO III (Endo sites) and the frequency of micronuclei in the buccal cells (MNBC). This study included 46 women from rural areas highly sprayed with pesticides and 101 controls from urban areas and without frequent use of pesticides in their homes. **Results and Discussion:** The average residence time was 28.00±8.00 years for women in rural areas and 29.77±2.55 years in urban areas ($p=0.871$). Both groups were similar regarding age, drinking and smoking habits, education level, and medicine consumption ($p \geq 0.155$). The results showed a significant inhibition ($p<0.01$) of AChE in women from rural areas compared to controls but no significant modifications in BChE. We also found an increase in DNA damage ($p<0.01$) and oxidazed pyrimidines ($p<0.001$) but no differences were found in the frequency of MNBC ($p=0.85$). When compared with previous results of men exposed to pesticide mixtures women showed a significant decrease respect to workers ($p<0.001$) but significant increase respect to men environmentally exposed to pesticides ($p<0.01$), using comet assay. In respect to oxidative DNA damage we observed significant increase in relation to both occupationally and environmentally exposed men ($p<0.01$ and $p<0.001$, respectively). Gender-sensitive research is needed to properly address the study of women's pesticide exposures and related adverse outcomes. **Conclusions:** A better understanding of potential gender–environment interactions related to pesticide exposure and health effects in women is needed, highlight the importance of developing strategies to intervene and mitigate pesticides exposure, which could potentially reduce the incidence of health effects.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 68- CHILDREN LEAD EXPOSURE FROM E-WASTE INFORMAL RECYCLING

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Introduction: Primitive electronic waste (e-waste) recycling is a source of exposure to a mixture of hazardous chemical contaminants, including lead. Population from urban low income neighbors are vulnerable, because metal and E- waste recycling represent a substantial economical family support. **Objective:** The aim of the present study was to examine lead exposure among children in low-income families who live around e waste open burning spots . **Methods:** A sample of children and adolescents with suspected exposure through e waste burning activities were assessed at the Environmental Pediatric Unit of the Department of Toxicology (University of the Republic) in Montevideo, Uruguay between November 2010 - July 2014. The sample included 69 children and adolescents. **Results:** Burning cables was the sole source of lead exposure in 28.9 % of the sample , BLLs within this group (n=20) was 8.23 ug/dl. Soil lead levels at the hotspots ranged from 650 to 19000 mg of lead per kg. Children living in areas with higher soil lead levels had significantly higher BLLs (r=0.50, $p < 0.01$). **Conclusion:** The results supports the fact that the e –waste open burning activities may be a significant source of lead exposure. Average children BLL in this study indicates the need for preventive interventions

EN 69- CYTOXICITY AND DNA DAMAGE BY PARTICULATE MATTER FROM BIOMASS COMBUSTION

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Objectives: Burning biomass may lead to significant increases in emissions of particulate matter (PM), which can have a serious impact on human health, and increase the risk of developing cancer, among other diseases. We performed this study because the International Energy Agency (IEA) promotes the utilization of clean biomass combustion applications to replace fossil fuels and reduce CO_2 emissions. The objective of this study was to characterize PM from different biomass fuels emitted from residential small scale applications and district heating plants. Furthermore, to investigate their effects on cytotoxicity and DNA damage in human lung cells in comparison to the two well-known cancerogenic substances nano-silica (nano-SiO_2) and diesel exhaust particles (DEP). **Material and Methods:** The investigated samples were obtained from two sources: Fly ashes from biomass power plants in St. Peter, Germany and Ammertzwiller, France, and PM from a laboratory boiler in Mulhouse, France. As fuel, wood chips and *Miscanthus* were examined. The particles were characterized by mineralogical and chemical techniques. They were investigated for their *in-vitro* cytotoxic and genotoxic effects (DNA alkaline unwinding assay) on human lung A549 cells and the immortalized human bronchial epithelial cell line BEAS-2B using submerse 2D culture and air-liquid interface conditions. **Results and Conclusions:** Fly ashes and PM from biomass combustion contain numerous solid chemical compounds such as quartz, cristobalite, various carbonates, halides and sulfates. The amount of PM emitted from automatic combustion power plants, especially those with electrostatic precipitator or cyclones, is very low. In contrast, residential small scale applications contribute to high ambient particle levels. Compared with nano-SiO_2, coal-fly ash (CFA) and DEP, the investigated fly ashes and PM from biomass combustion showed only weak genotoxic effects (~ 100 µg/cm²). CFA and DEP have significant DNA-damaging effects, even at very low concentrations (10 µg/cm²). PM from *Miscanthus* showed no significant cytotoxicity or genotoxicity up to concentrations of 100 µg/cm². From our results we conclude that biomass-fueled heating especially with *Miscanthus* may be a good alternative to heating with coal or other fossil fuels. Thus, if biomass is to be used as a fuel in future, toxicological information on the chemical and physical properties of the fine particles emitted should be further investigated in order to assess the public health risks (especially cancer) and to give suitable recommendations for future scenarios with increased biomass combustion.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 70- IN VITRO GENOTOXICITY ASSAYS FOR ENVIRONMENTAL MONITORING AT THE UPPER PARANA RIVER WATERS (MISIONES, ARGENTINA)

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The Paraná River is the second most important drainage basin in South America and concentrates some of the major urban and industrial areas from Brazil and Argentina through its course. This freshwater system has received special attention due to the intensive anthropic modifications suffered. In vitro assays based on mammalian cell lines are commonly used in genotoxicity test and can offer an interesting alternative in ecotoxicology studies. The present work proposes to evaluate the genotoxic and cytotoxic potential of water from four different sites along the Upper stretch of Parana River (Misiones Province, Argentina) during the period 2009-2011. For this purpose, were applied the in vitro micronucleus (MN), nuclear alterations (NA) and comet (CA) assays by exposing CHO cells during 2 hours (CA) and 15 hours (MN and NA). The mitotic index (MI) was calculated to assess the cytotoxicity. Positive and negative controls were performed employing 0.4 mM of Methilmetanesulphonate and PBS solutions respectively. Simultaneously, a Water Quality Index (WQI) was established for each sample site and the results obtained for all treatments were compared using ANOVA and Kruskal-Wallis test. Genotoxic effects were detected in all cases, both the MN/NA and CA assays showed the higher DNA damage over the last two years (2010 and 2011). Statistical differences ($p < 0.05$) from the negative control were observed in almost all sampling sites and these data correlate with lower values of WQI and MI. Water samples from the two sites that receive effluents from paper mill industries promoted the higher DNA damage level detected with both techniques. Only the site that receives wastes without any treatment system showed statistical differences in relation to the control in all studied years. The other two sample sites, northern and southern, induced lower damage level. Complex mixtures of paper mill industries and urban wastes in Upper Paraná River waters could induce direct damage potentially repairable as detected by comet assay, as well as other DNA damage evidenced by the presence of MN, nuclear buds and nucleoplasmic bridges associated to the presence of substances that induce aneugenic and clastogenic effects. The in vitro assay using CHO cells showed high sensitivity to DNA damage after exposure to the different water samples with both genotoxicity tests and can represent a suitable alternative for environmental monitoring of freshwater bodies. Genetic biomarkers like MN, NA and CA represent sensitivity early-warning signals in response to pollution and constitute a good complementary tool with physicochemical analysis for an integral evaluation of aquatic environments.

EN 71- IDENTIFICATION OF FORMS OF PESTICIDES STORING AND EMPTY PACKAGES' DESTINATIONS IN RURAL TOBACCO GROWING PROPRIETIES IN THE STATE OF PARANÁ

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Introduction: The use of pesticides in the country has led to many concerns in the society and health sector; due to its high consumption rate it leads the world ranking. Therefore, the ways in which pesticides are stored as well as the disposal of empty pesticides packages, can represent an environmental and human health contamination risk taking into account its toxic potential. **Objective:** The objective of this article is to present the results of the environmental inquiry completed in 142 rural tobacco growing proprieties in the State of Paraná, where were identified the pesticide storages and packages disposal. **Materials and Methods:** Were selected 15 proprieties in each of the 10 counties considered high-priority in tobacco production, then was applied a questionnaire to raise data related to risk factors, which were then digitalized into the Ministry of Health's DATASUS, named FORMSUS. **Result and Discussion:** The inquiry was realized in 142 rural tobacco growing proprieties which uses pesticides in their method of production. Of these proprieties, 128 (90%) possess a specific location for the storage and pesticides, and 99 proprieties (70%) even have safety parameters such as locks. It was stated that regarding the organizing of the pesticides only 34 proprieties (24%) did actually organize, and that in 77 proprieties (54%) there was a good ventilation system. In 115 proprieties (81%) the artificial light was considered adequate and that in 99 proprieties (70%) the equipment for pesticide pulverization had specific storage locations. In regards to the disposal of the pesticides packages in 74 proprieties (52%) they were kept for a short period of time in the same area as the other chemicals used in the propriety and in 11 proprieties (8%) there was a specific location for all empty packages. It was noticed that the reuse of empty packages for other ends occurred in 18 proprieties (13%). **Conclusion:** In order to improve the conditions in which pesticides and their empty packages are stored, actions to educate tobacco growers on these matters must be done, such as elaborating and distributing educative pamphlets and subsiding health groups with the single purpose of teaching. It is also needed that an inter-department relation is created, to ensure the promotion of human and environmental health in regions of tobacco production, in the state of Paraná.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 72- EFFECTS OF THE ROUNDUP EXPOSURE: CHOLINESTERASE ACTIVITY IN THE POLYCHAETE *LAEONEREIS ACUTA* (NEREIDIDAE)

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Introduction: ROUNDUP consists in a formulation of glyphosate (active ingredient) and surfactants. This herbicide is commonly used in agriculture and vegetation control since glyphosate inhibits the enzyme 5-enolpyruvylshikimate phosphate synthase responsible for the aromatic amino acids biosynthesis. Some ecotoxicological studies reported harmful effects to aquatic animal physiology including changes in the antioxidant capacity, ROS generation, lipid peroxidation as well as trough enzymatic inhibition, like acetylcholinesterase activity. **Objective:** The objective of the present study was to analyze the ROUNDUP effects on cholinesterase activity in the polychaeta *Laeonereis acuta*. **Material and Methods:** The worms were collected in Saco do Justino, Southern Brazil and acclimated in the laboratory conditions for 7 days (20 °C; salinity 10, pH 8.0, 12L:12D). They were divided into 3 groups (control and two different ROUNDUP concentrations - C_1=3.25 mg/L and C_2= 5.35 mg/L) for 24 h and 96 h. These concentrations represent the NOEC and CL10 96h for this animal, respectively. After that, pools with the anterior region of 4 animals were homogenized with sodium phosphate buffer 20 mM plus 20% glycerol and centrifuged (9,000 x *g*, 4°C for 30min). The supernatant was employed as the Soluble Fraction (SF). The pellets were ressuspended using the previous buffer added with Triton X-100 0.5%following the same centrifugation step and this supernatant was employed as membrane fraction (MF). The colinesterase activity (mmoles/mg prot/min) including the acetylcholinesterase (AchE) and propionilcholinesterase (PchE) was measured following the Ellman method using dithiobisnitrobenzoic acid (DTNB) that reacts with thiocoline which absorbance is captured at 412 µm. The results were analyzed using one-way analysis variance (ANOVA) and Newman-Keuls test ($p<0.05$). **Results and Discussion:** The AchE and PchE activity showed significant differences at 96 h exposure in MF. AchE and PchE was inhibited aproximately 28% comparing with the control group. AchE values were 0.071±0.005 (3.25mg/L) (); 0.072±0.005 (5.35mg/L) and 0.107±0.009 (control). PchE values were 0.066±0.004 (3.25mg/L); 0.063±0.004 (5.35mg/L) and 0.09±0.00 (control). No significant differences were observed in MF neither SF at 24 hours ($p>0.05$). These results corroborates with previous studies that reported colinesterase activity reduction in muscle and nervous system of fish and mollusks. **Conclusion :** The present study confirmed the ROUNDUP toxicity on *L.acuta* colinesterase. The cholinesterase assay can be used in the worm to determine the effects of xenobiotics like herbicides.

EN 73- DRUG COLLECTING PROJECT IN THE CITY OF PALMAS-TO

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Introduction: The presence of drugs in natural resources has been causing concern in ecotoxicological studies because the traditional processes of collection and treatment of wastewater do not remove or inactivate these biologically active metabolites. Between 50% to 90% of ingested drugs are excreted in the urine in addition to the disposal of anthropogenic source in the sewer and trash. As a consequence impacts on public and environmental health, thus preventive and educational action minimizes home toxicity and biological hierarchy, for that the proper disposal of medicines. **Objective**: Give a proper destination for the underutilized medicines; inform and educate the community about proper disposal. **Materials and Methods**: continuous educational action was carried out in the university community (CEULP / ULBRA), with students from public and private schools and professionals from community pharmacies. The collection of expired and obsolete drugs were in public and private institutions partners of the project. The drugs were stored in a safe and suitable location. Later each unit was registered by active ingredient, concentration, dosage form, quantity and therapeutic class; then separated and sent for optimal final disposal: incineration of the drug and recycling of carton and label. **Results and Discussion**: 4929 medicines were collected between the years 2013 to 2015, the year 2015 refers to the first semester. In 2013 they collected 2132, in 2014 collected 2324, and the first semester of 2015 were 1473. There was growing collection due to disclosure and new project partnerships. It was observed that antihypertensives and diuretics are the most classes collected in 2013 and 2014, corresponding to 49.48% and 23.87%, respectively, while in 2015, with 28.12% antimicrobial. The contraceptive class amounted to the second highest percentage in the years 2013, 2014 and 2015, which means, 11.92%, 19.42% and 22.83%, respectively. In 2013 the median portion was represented by antimicrobial 5.78%, glucocorticoids 4.51%; in 2014 by antimicrobial 10.41%; in 2015 by anti-inflammatory 12.90% and analgesic 10.36%. Antimicrobial natural resources favor the resistance of environmental microorganisms changing biogeochemical cycles and hormones affect the reproductive system of aquatic organisms. The result of these and other interactions in the medium and long term is a current concern in ecotoxicological studies, particularly of sensitive analytical methods because they are invisible and dangerous pollutants. **Conclusion**: Most of the population does not know what the correct disposal of expired and obsolete drugs in addition to ignoring the environmental impacts. This fact makes clear the importance of adopting educational campaigns in partnership with health professionals.

Acknowledgements: Our thanks to CEULP / ULBRA for giving the opportunity to carry out this project. We also thank Professor Emilia Jacinto Trinity, our advisor, by stimulating the development of this work and for valuable discussions during the same. In order to cope with the needs and challenges that arise in the face of environmental toxicology.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 74- METALLOMICS AND PROTEOMICS REVEALS A COOPERATIVE MODE OF ACTION IN MANGANESE-INDUCED NEUROTOXICITY ON PRIMARY CEREBELLAR GRANULE CULTURE.

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Introduction: Manganese (Mn) is essential for living organisms, playing an important role in nervous system function, bone mineralization, protein and energy metabolism, metabolic regulation and cellular protection. Nevertheless, chronic and/or acute exposure for this metal, mainly during early life stages can lead to neurotoxicity and dementia (cognitive and neurobehavioral impairment) whithout clear mechanisms[1]. For that reason, we hypothesed that the complexity and unsolved mechanism of the neurotoxicity induced by manganese can be associated with the activation of simultaneous and/or concurrent pathways, which can be dependent of chemical speciation too. **Objective:** Therefore, this study was to investigate the mechanisms mediating the toxic effects of MnCl2, Maneb and Mancozeb in primary cultures of cerebellar granular (CGC) neurons. **Material and Methods:** Cell viability was verified by MTT assay[1]. The metal homeostasis (metallomics) was verified by by inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 730 - Perkin Elmer)[2]. The proteomic study of primary cell culture exposed for chemical species of Mn was conducted according to Thermo Scientific Tandem Mass Tag (TMT) protocols[3]. Finally, biostatistics analysis were performed according to Hernández et al[1]. **Results and discussions:** Despite, we verified that both maneb and mancozeb induced the same degree of neurotoxicity as well as chronical exposure of cerebellar granule neurons from early to lates differentiation stages is more important than acute exposure like previous works of our group, using $MnCl_2$, these pesticide appear to be show different mode of action than $MnCl_2$. We verified that this can be by cooperative pathways, associated with metal dyshomeostasis, energy metabolism impairment, oxi-reductive stress and other effects that lead to apoptosis, compromising the normal neuronal development. However, we indentified that contrarily to $MnCl_2$, the Maneb disrupted the pathway associated with huntington's disease. **Conclusion:** These findings suggest that Mn-induced developmental neurotoxicity of manner chemical specie dependent, including different mode action.

References:

1. Hernández, R.B., Farina, M., Espósito, B.P., Souza-Pinto, N.C., Barbosa, Jr, F., Suñol, C., 2011. Toxicol. Sci. 124(2): 414-423

2. Hernández, R.B., Nishita, M.I, Espósito, B.P., Scholz, S., Michalke, B. 2015. Journal of Trace Elements in Medicine and Biology 32, 209-217.

3. http://planetorbitrap.com/tmt#.VJe-OXkAand

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EN 75- IDENTIFYING ENVIRONMENTAL RISKS FROM LEAD EXPOSURES THROUGH THE USE OF A QUESTIONNAIRE AT THE PRIMARY CARE LEVEL.

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INTRODUCTION: The Pediatric Environmental Health Specialty Unit in Uruguay (PEHSU-U) developed a short questionnaire for the Primary Health Care level. This questionnaire contains identifying data and ten “yes” or “no” questions directed at exposures to urban environmental risks around homes (with special interest in lead exposures). **OBJECTIVE**: To evaluate if the community members perceive the environmental exposure risks to lead in and around their homes, related to blood lead levels (BLL) identified among vulnerable populations (pregnant women and children). **MATERIALS AND METHODS:** A population-based study of a suspected lead contamination area of Montevideo. A short questionnaire and BLLs were analyzed in Epidata using the following variables: age, gender, answers to the questions, and BLLs. The blood samples were taken by PEHSU-U staff using a finger prick and analyzed with Lead Care II. **RESULTS & DISCUSSION:** The majority of patients interviewed (140) were female (56%); with an average age of 12.9 years (0.5 to 59 years old; SD 0.96). Only 126 had a BLL test, with an average of 4.51 µg/dL (3.0 to 28.1; SD 6.06). The Blood Lead values show: Group 0-4.9 µg/dL (n=91) represents 72% of the population studied; group 5 to 9.9 µg/dL 12%; group 10-19.9 µg/dL 10%; and more than 20 µg/dL 6%. We found statistically significant associations between BLLs > 5 µg/dL and identification of in-home lead exposure in 3 out of 6 questions related to direct or indirect lead exposure. The questions were about old and peeling paint (questions 4 p=0.029); recycling and scrapping metals (question 6 p=0.007) and employment with metals in a family member (question 7 p=0.041). No association was found between BLL and questions regarding exposures outside their homes, soil contamination (question 1), open garbage and wire burning (question 3) and contaminated family member or neighbor (question 2) . **CONCLUSIONS**: The short questionnaire may identify the risks to indoor lead exposure (recycling and scrapping metals; old and peeling paint; and employment with metals in a family member). This positive association , may be sensitive enough to help health professionals at primary health care level to identify children with more than 5 µg/dL the reference level of the national follow up protocol . Further studies are necessary in order to confirm this results.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 76- COMPARATIVE STUDY OF TOXIC-GENETIC EFFECTS OF NATURAL RADIOACTIVITY IN DIFFERENT LINES OF *Drosophila melanogaster* RATED BY COMET ASSAY

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Introduction: The presence of radionuclides such as Uranium is a common reality in the semiarid region, Rio Grande do Norte state (RN), Brazil. Being the Uranium an unstable atom, undergoes a process of decay producing several decomposing products, including Radon gas. This compound released into the atmosphere is associated to various pathological processes and elevated cancer risk, especially in the municipality of Lajes Pintadas/RN. Among the tests able to detect genetic damage induced by different genotoxic agents highlights the comet assay, widely applied in environmental and human biomonitoring. The use of *Drosophila melanogaster* model in genotoxic tests have been increased thanks to the ease of his laboratory handling, rapid phenotypes detection and deep knowledge of its genome. **Objective:** This study aimed to investigate the genotoxic effects of environmental radiation present in Lajes Pintadas/RN in hemocytes of *Drosophila melanogaster* larvae from two different lines: Oregon-R and Wild strain. **Material and Methods**: *Drosophila melanogaster* larvae were exposed during six days in the natural environment of Lajes Pintadas/RN until they reach the third instar. The hemocytes of 180 larvae cells were isolated and subjected to adjusted comet assay methodology. DNA damages visualized in comet assay were classified into five classes (0-4) and from these data were calculated the Damage Index (ID) and the Damage Frequency (FD %). Two strains were exposed in the field: Oregon-R, one standard line used in genotoxic tests, and the Wild strains, gathering in the city of Recife/Brazil. A positive control group (treated with Cyclophosphamide to validate the comet assay) and a negative control (untreated, to compare the results of treatments in the field) was established in laboratory conditions, for both strains. The Kruskal-Wallis and Mann-Whitney tests were used in statistical analysis ($p \leq 0.05$). **Results and Discussion:** The results showed a significant increase in FD and ID% for two strains exposed to natural radiation, Oregon-R ($p = 0.025$) and Wild strain ($p = 0.025$), compared to their respective negative controls. Therefore, there was an increase in genetic damage in the most studied strain in the literature, Oregon-R, as in the Wild strain. **Conclusion:** The results validate the use of natural populations of *D. melanogaster* in the comet assay and prove that the method is efficient and sensitive to detect genetic damage caused by natural radiation associated with Radon gas. The results also confirm other studies made with human populations in Lajes Pintadas/RN, and pave the way for the applied use of *D. melanogaster* in environmental biomonitoring.

EN 77- COMET ASSAY SENSITIVITY IN ADULT SOMATIC CELLS OF *Drosophila melanogaster* EXPOSED TO DIFFERENT GRADES OF AIR POLLUTION

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Introduction: Air pollutants are directly related to the phenomenon of urbanization, a process of relative growth in a country's urban population relative to rural areas. Pollutants produced by this phenomenon are a risk for genomic integrity in all living organisms because of its great potential to damage the genetic material, partly induced by oxidative stress, to a lesser or greater degree. This genetic damage represents a risk to the appearance of many types of cancer, physiological and degenerative diseases and other harmful consequences. Application of the comet assay in *Drosophila melanogaster* model has become a promising tool for the investigation of genotoxic activity, being an important device of biomonitoring. **Objective:** The objective was to apply the methodology of the comet assay in hemocytes of adults of *D. melanogaster* testing it´s potential for the detection the genotoxic effects of air pollution associated with different grades of urbanization. **Material and Methods:** Hemolymph of adult cells, rather than larvae cells, of a Wild strain of *D. melanogaster* were studied after exposure of the individuals in two areas with varying levels of pollution: a large urban center (in Recife, capital of Pernambuco, Brazil), and a remote rural area 19.7 km apart (District of Aldeia dos Camarás, Pernambuco). After six days living in population boxes in the field, the hemocytes of 180 adult cells were isolated and subjected to adjusted comet assay methodology. DNA damages visualized in comet assay were classified into five classes (0-4) and from these data were calculated the Damage Index (ID) and the Damage Frequency (FD %). The efficiency of the comet assay in somatic cells from adults was corroborated by the positive control group established in the laboratory, treated with the Cyclophosphamide. The Kruskal -Wallis and Mann-Whitney test ($p \leq 0.05$) were applied to the results. **Results and Discussion:** The results showed a significant increase in ID and FD% of both exposed groups, Recife ($p = 0.023$) and Aldeia dos Camarás ($p = 0.025$), compared to negative control group (established under laboratory conditions). However, there were no significant differences between the two areas surveyed ($p = 0.061$). Most likely, the two locations have the same levels of air pollutants because the Recife point is situated 300 m from the Atlantic Ocean (with high level of air circulation and dispersion of pollutants) and the rural place is located 10 km apart of a thermoelectric plant, which may be contributing to an increased pollution index. **Conclusion:** The results open new perspectives for the use of somatic cells from adults of *D. melanogaster* tested for a first time, as a sensitive and useful *in vivo* model to detect genotoxic damage in different scenarios of environmental air pollution.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

EN 78- RADIORESISTANCE IN NATIVE BRAZILIAN *Drosophila melanogaster* AVALIATED BY COMET ASSAY, A SENSITIVY GENOTOXIC TEST

CASTRO I.F.A.[1,2], VERÇOSA C.J.[1], SANTOS R.G.[2], SANTANA S.L.[2], SILVA A.S.[2], AMORIM E.M.[2], NAVONI J.A.[3], AMARAL V.S.[3], ROHDE C.[2]

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Introduction: Living organisms receive large amounts of radiation and much of them come from natural sources. Rio Grande do Nortestate in Brazil has higher levels of natural radiation, especially in the Lajes Pintadas city, rich in Uranium, Thorium and Potassium. Uranium is an unstable chemical element and its decay produces various sub products, including Radon gas that is associated with numerous cancers, especially in the respiratory tract of humans. As a result of continued exposure, native organisms can adapt and develop radioresistance mechanisms compared to non-native groups. **Objective:**Compare the genotoxicity in larvae cells of two strains of *Drosophila melanogaster*, one maintained for decades in the laboratory (*Oregon-R*) and one native strain (collected in the city of Lajes Pintadas), in order to evaluate the possibility of natural adaptation to the radiation. **Material and Methods:**Native strains of *D. melanogaster* were collected in July/2014 in Lajes Pintadas and were maintained since them in the laboratory. Six months later (January/2015) and 12 months later (July/2015), descendants returned to Lajes Pintadas, where they remained for six days, until the larvae reached the third instar. Descendants of *Oregon-R* strain, sensitive to natural radiation, were also exposed in Lajes Pintadas. An environmental negative control group, also *Oregon-R,* was exposed to the preserved area Catimbau National Park, 400 km apart. Hemocytesfrom 180 larvae of each experiment were extracted and subjected to the comet assay procedure. The comets were visualized and classified into five grades (0 to 4) and from these data were calculated the Damage Index (ID), and the Damage Frequency (FD%), analyzed by ANOVA and Bonferroni statistical tests. **Results and Discussion:**Significant differences for ID were observed between exposed natives and *Oregon-R* exposed to radioactivity conditions of Lajes Pintadas, in January/2015(p=0,001) and in July/2015 (p=0,001). FD% the results werealso statistically different between natives and *Oregon-R*in July/2015 (p=0,001), but were not for the January/2015 (p=0,244). The native fliesfrom Lajes Pintadas also behaved in the same way as the *Oregon-R*flies exposed to Catimbau with no statistical differences for ID and FD% (p=0.002 and p=0.003). **Conclusion:**The results here presented are the first indication that populations of the *D. melanogaster*living in Lajes Pintadas can be resistantto genotoxic effect caused by natural radiation. The native strains presentedless indices and frequency of DNA damage than non-native strains. Natives flies of Lajes Pintadas can support the natural radioactivity and present the same low levels of DNA damage found in other non-radioactivity and conserved environments, like as Catimbau National Park.

EN 79- *IN VIVO* GENOTOXICITY EFFECT OF NATURAL RADIATION AVALIATED BY COMET ASSAY IN *Drosophila melanogaster* IN TWO MUNICIPALITIES OF NORTHEAST BRAZIL

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Introduction: Uranium (U) is a chemical element with natural occurrence. This usually is located inside the earth's crust, but natural and anthropogenic processes can contribute to its redistribution throughout the environment. In its decay chain is produced the Radon gas one of the main responsible for rise of cancer and other pathological processes. In the toxicological genetic area, Comet Assay figure as a useful tool for human and environmental monitoring and can be applied to diverse organisms, including flies of genus *Drosophila*. **Objective**:The aim of this study was analyze the DNA damage caused by exposure of *Oregon-R*strain of *Drosophila melanogaster* in the cities of Santa Cruz and Lajes Pintadas, distant 17km form each other, in order to measure the sensitivity of the flies to different atmospheric radiation levels. **Material and Methods**:*Drosophila melanogasterOregon-R*strains were exposed during six days inside the appropriate population boxes (in three replicates) in the field of two cities, Lajes Pintadas and Santa Cruz (Rio Grande do Norte state, Brazil), during the dry (January/2015) and rainy season (July/2015). As a negative environment control, the same strain was exposed inthe Catimbau National Park, located in Buíque, Pernambuco state, 400 km apart. Hemocytes of 180 larvae were submitted to comet assay, and the results (comets) were classified into five classes (0 to 4). From these data were calculated Damage Index (ID) and the Damage Frequency (FD %) evaluated by ANOVA and Bonferroni statistical tests. **Results and Discussion**: Statistical analysis revealed significant differences between the exposed groups Lajes Pintadas and Santa Cruz, in dry and rainy season for ID (p=0.001 for both) and in rainy season for FD% (p=0.001). The highest value of genotoxicity observed in exposed flies in Lajes Pintadas wasan expected result, since the city is known to have high concentrations of Radon (Rn) in the air, and high cancer incidence in residents. But the low genotoxic effect in flies exposed in Santa Cruz was a surprise, because both cities are situated very close (17 km) and because previously radiation measurements (*data not published*) indicate levels of Radon in the air higher than 300 Bq/m^3. Compared to the negative environmental control group the ID and FD% obtained in Santa Cruz were not significantly different. And, as also expected, genotoxicity levels of Lajes Pintadas were higher than Catimbau, in dry and rainy season for ID (p = 0.004, p = 0.002) and in rainy season for FD% (p = 0.002). **Conclusion:** The results show that the comet assay with hemocytes of *D. melanogaster* larvae was very sensitive in the detection of different levels of genotoxic among the three sites studied (Lajes Pintadas, Santa Cruz and Catimbau National Park). It also reinforces previously studies indicating that exposure in the city of Lajes Painted present risks to the genetic material of organisms.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

NANOTOXICOLOGY

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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NT 01- OXIDATIVE STRESS AND CELULAR ALTERATIONS IN DIFERENT TISSUES OF *Litopenaeus vannamei* INDUCED BY GRAPHENE EXPOSURE

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Introduction: Graphene is a nanomaterial formed by a carbon layer (2D) with a sheet size and honeycomb arrangement, this way it tends to aggregate on cells surface and may alter cellular properties. In the aquatic environment, nanomaterials tend to aggregate in the sediment endangering organisms living, for that reason graphene has a toxicological potential, considering that living organisms have the capacity to incorporate toxic particles present in the environment. The aim of the study was to evaluate the effects on *Litopenaeus vannamei* given the exposure to graphene supplemented in the diet, and if it can induce oxidative stress scenario. **Methods**: Animals were divided into a control group (n=8) and treated group (n=8), the control group was fed with feed and the treated group exposed to a concentration of 500mg/kg of graphene supplemented in the feed. This procedure was performed in two different times; after four weeks of exposure, animals were euthanized by freezing and in the first exposure hepatopancreas, gills and muscle were dissected to determine the reactive oxygen species (ROS) levels in fresh tissues. Subsequently, the remained parts of these tissues were used to perform the follow analysis: glutamate cysteine ligase (GCL) enzyme activity, reduced glutathione (GSH) levels, glutathione-S-transferase (GST) activity, total antioxidant capacity of tissues and peroxidation lipid levels. In the second exposure a total of 12 animals were used, 6 in with group, after the euthanasia histological procedures were executed with hematoxylin and eosin in the same tissues. **Results/Discussion**: Results showed responses to graphene exposure by increasing ROS generation in hepatopancreas and gills besides altering their antioxidant defense system, since there was an increase in the GSH concentration as well in the total antioxidant capacity; also an increase of GCL activity in hepatopancreas and decrease in the gills, in addition opposed results were found in GST activity, with increase of GST activity in the hepatopancreas and a decrease in the gills. In these tissues, however, were found lipid oxidative damage. Also in hepatopancreas hyperplasia were observed in basal cells and decrease in secreting cells. There data available concern *in vitro* studies, showing that in mammalian and bacteria cells exposure to graphene induced ROS production besides a change in GSH concentration resulting in alterations on the redox state of the cells. **Conclusions**: The results suggest that graphene is a pro-oxidant agent when the organism is exposed through diet, inducing oxidative stress due to increased ROS and antioxidant system modulation and pathology in hepatopancreas, causing changes in physiological processes and jeopardizing health of the animal.

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NT 02- CYTOTOXICITY OF CLAY-TiO_2 NANOSTRUCTURES IN HUMAN HEPG2 CELLS.

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Introduction: In the last decades, nanotechnology has become increasingly attractive to different scientific and industrial fields as it becomes evident that manufactured nanomaterials offer a whole new range of potentialities. On the other hand, different studies have been showing that nanomaterials can be toxic and therefore may harm both the environment and the human health. Recent advances in the production of nanomaterials lead to the development of new structures, namely of nanoparticles immobilized in microstructures that by presenting new physico-chemical features must be test in regards to their toxic potential. **Objective:** In this context, the main objective of this work is to evaluate in vitro cytotoxicity of TiO_2 immobilized in clay (C-TiO_2) in a hepatocellular carcinoma human cell line (HepG2). In order to understand the origin of the observed effects, TiO_2 and clay alone will also be studied. **Materials and Methods:** Materials were supplied by the Ceramic for Smart System Group of the Electroceramic Department, Instituto de Ceramica y Vidrio, Madrid, Spain and characterized by dynamic light scattering (DLS) for particle size, particle distribution and suspension stability and scanning electron microscopy (SEM) for particle size distribution. After cell exposure to different concentrations and time periods, cellular viability was assessed by employing MTT and Alamar Blue (AB) assays. Experiments with materials dispersed in complete and incomplete medium were conducted in parallel to understand the possible influence of serum presence in the observed toxicity. **Results and Discussion:** TiO_2 induced a significant dose-dependent decrease in viability of HepG2 cells particularly visible in complete medium, both in MTT and AB assay. Although clay minerals have been previously referred as non-toxic, the tested clay nanoparticle induced significant cell death of hepatocellular carcinoma human cells, for both types of medium and for the majority of the time frames tested in these experiments. A similar trend was observed for C-TiO_2, probably due to the fact that this structure is mainly composed by clay (90%). **Conclusions:** These new nanostructures are undoubtedly promising for different and important applications in the biomedical field. As new nanomaterials are currently being produced and introduced in the market, it is of paramount importance to evaluate their potential toxicity. Results here obtained show that C-TiO_2 nanostructures seem inappropriate for biological and biomedical applications since these structures do not improve the biocompatibility of single TiO_2 in hepatic cells, but, in opposition, increase their toxicity.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

NT 03- BIOCHEMICAL RESPONSES INDUCED BY CO-EXPOSITION TO ARSENIC AND TITANIUM DIOXIDE NANOPARTICLES IN THE ESTUARINE POLYCHAETE *Laeonereis acuta.*

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Introduction: Nanomaterials (NM) are widely used for various applications and the release of this NM in the environment seem be inevitable. The NM titanium dioxide ($nTiO_2$) is extensively produced and used due their specific properties, besides being applied for the water decontamination of metalloids such as arsenic (As). As is a contaminant commonly distributed in the environment to which humans are routinely exposed through various routes of exposure. Although some studies have shown the low toxicity to aquatic animals of $nTiO_2$, it is known that As can induce various deleterious effects, including oxidative stress, however, few studies have demonstrated the potential toxicity of these two compounds together. The interaction of NM to contaminants, as As, may potentiate the toxicity of the contaminant and/or their accumulation in different cells and tissues, effect known as a Trojan horse. **Objective:** The objective of this study was to evaluate if the co-exposure of $nTiO_2$ and As may influence the toxicity of As in the estuarine polychaete *Laeonereis acuta* (Nereididae). **Materials and Methods:** The animals were exposed to 50 µg/L of arsenite ($NaAsO_2$) and/or 100 mg/L of $nTiO_2$ during two days. After exposure the animals were homogenized, the homogenates were centrifuged and finally the supernatants were removed for the realization of biochemical analysis such as reactive oxygen species (ROS) levels, reduced glutathione (GSH), glutathione-S-transferase (GST) and glutathione reductase (GR) activity, total antioxidant capacity, lipid peroxidation (TBARS) and DNA damage. The data obtained in dosages were tested using analysis of variance (ANOVA) followed by Tukey test (α= 0.05). **Results:** There was increase in ROS levels in the group exposed simultaneously to As + $nTiO_2$ and decrease in total antioxidant capacity in the same treatment. GSH levels and GST activity were not altered in any treatment while in the group co-exposed was observed an increase in GR activity beyond lipid and DNA damages. **Discussion:** Previous studies showed that As induces the modulation of antioxidant system and oxidative stress situation in *L. acuta.* Few studies are considering the toxicity of NM in aquatic organisms and the interaction of them with other contaminants as As. In our study was possible to observe that the co-exposure to As + $nTiO_2$ induced responses not observed after As exposure alone, suggesting the harmful effect of NM together with other environmental contaminant. So, the use of $nTiO_2$ to remove As from contaminated water seem a doubtful alternative once that could jeopardize the aquatic biota. **Conclusion:** From the results obtained in this study, was possible to observe that the co-exposure to As and $nTiO_2$ induced a stress oxidative situation through of Trojan Horse effect.

NT 04- HEPATIC TOXICITY OF TITANIUM DIOXIDE NANOPARTICLES IN FISH: STUDIES ON *Hoplias intermedius*

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Nanomaterials are an important product of nanotechnology and are coming into use in healthcare, electronics, cosmetics, and other areas. This field of technology has been raising quickly but the knowledge about the potential harmful of nanomaterials to the environment has been subestimated, because they have been released into the environment in the absence of regulations. A growing number of civil society organizations worldwide have called for precautionary management of nanotechnology. All manufactured nanomaterials must be subject to safety assessments as new substances, even where the properties of their larger scale counterparts are well known. The aim of this study was to assess the hepatic toxicity of titanium dioxide (TiO_2) nanoparticles on a fish specie *Hoplias intermedius*, and the interaction with metals by co-exposure. The fish were intraperitoneally injected with 0.1, 1.0 and 10 μgg^{-1} TiO_2, 21 μgg^{-1} lead (Pb), 50 μgg^{-1} aluminum (Al), and the mixture of each dose of TiO_2 with the two metals. A negative control was carried out. After 96 h the liver was taken up for comet assay and ethoxyresorufin-O-deetilase (EROD) and glutationa-S-transferase activities. The physical-chemical description of the colloidal suspension by Zetasizer® was investigated. The colloidal suspension 0.01 and 0.1 $mgmL^{-1}$ had a mean size 338 and 137.5 nm respectively. The 1 $mgmL^{-1}$ suspension had two peaks of particle size: 1782 and 5105 nm. Percentage of polydispersion was higher than 20% for all suspensions, so they were heterogeneous about particles size. All colloidal suspensions had negative zeta potential (between 0 and -30), and they were unstable. The results showed no evidence of TiO_2 genotoxicity. DNA damage were observed after lead and aluminum administration. The EROD activity did not change in the groups treated with TiO_2 compared to control group. It was observed an increase in the activity after aluminum treatment. When the mixtures are considered, just $0.1\mu gg^{-1}$+Pb had higher EROD activity than control, besides all the TiO_2+Al doses. Regarding GST activity the lower doses of TiO_2 decreased this enzyme activity, while Pb increased it. The mixture of 0.1 and 10 μgg^{-1} of TiO_2+Al decreased the enzyme activity. It was possible to observe interaction between 10 μgg^{-1}+Al, since GST activity was lower compared to the groups 10 μgg^{-1} and Al. The TiO_2 nanoparticles can alter early biochemical defenses, like xenobiotic metabolism enzymes, both phase I and phase II. These are preliminary studies, so we are still looking for tissue-specific toxicity and investigating the risk assessment by other biomarkers.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

NT 05- SAFETY OF SHORT-TERM TREATMENT OF SILVER NANOPARTICLES AGAINST ENTEROPATHOGENIC INFECTION OF SALMONELLA TYPHI IN RATS; IMMUNOMODULATORY EFFICACY STUDY

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Introduction: Salmonellosis-induced diarrhoea is one of the commonest cause of childhood mortality in developing countries. *S. typhi* strains showed resistance against various antimicrobial agents as β-lactams, quinolones, and aminoglycosides. Silver nanoparticles (AgNPs) has been attracted scientist due to its various applications, including photonic devices, biosensors, antimicrobials, and drug delivery systems. The antibacterial effect of AgNPs offers opportunity to eradicate the bacterial infections and overcome resistance that induced by *S. typhi*. **Objective:** the current study aims to investigate the effect of silver nanoparticles (AgNPs) against salmonella infection. **Materials and Methods:** Four groups of female Sprague-Dawley rats were treated for eight days as follows: group (1) untreated control; group (2) was challenged with single inoculation S. typhi, and groups (3) were treated with AgNPs (100 mg/kg) for seven days, respectively. Group (4) was challenged with S. typhi at day one, and then treated with AgNPs (100 mg/kg) for seven days. At the end of treatment period samples of small intestine and liver were collected from each group for histological examinations. **Results and Discussion:** Salmonellosis induced sever changes of immunological pattern; IgM, IgG, IgA and ALP, AST, ALT. On the other hand, silver nanoparticles succeeded to eradicate typhoid infection, restore the values of immunological parameters and biochemical data to typical levels of control group, and improve histological pictures of intestinal and hepatic tissues. **Conclusions:** It can be concluded that silver nanoparticle (AgNPs) are promising and safe candidate in treatment bacterial infection induced by S. Typhi due to its antimicrobial, and immunomodulatory activities.

NT 06- INVESTIGATION OF THE GENOTOXICITY OF TIO2 NANOPARTICLES IN A549 CELLS

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Introduction: Metal oxide nanoparticles (NPs) especially titanium dioxide is being used increasingly for various cosmetics (especially sunscreens), food and drug products. Because of widespread environmental exposure to metal oxide nanoparticles, it is urgent to elucidate their effects on human health. Recently, various toxic effects of NPs have been widely investigated in cell lines using different endpoints. On the other hand the results of these studies are not compatible with each other. **Objective:** The aim of this study is to investigate the toxic effects of three different nanosize (20 nm, <100 nm and <150 nm) of TiO2 NPs by in vitro toxicological studies. The cytotoxic and genotoxic effects of TiO2 NPs were investigated in A549 cell line (adenocarcinomic human alveolar basal epithelial cells). **Materials and Methods:** Cytotoxicity of TiO2 NPs (up to 100 µg/mL) were tested by using Neutral Red Uptake (NRU) assay. For testing the genotoxic effects of TiO2 NPs (5, 10, 50 and 100 µg/mL for each particle sizes), comet assay was used in A549 cells. **Results and Discussion:** The tested concentrations of TiO2 NPs sizes of 20 nm, <100 nm and <150 nm by using NRU assay has no cytotoxic effect. However at 20 nm particle size of TiO2 NP's IC40 were calculated at 100 µg/mL concentration. Comet scores obtained from TiO2 NPs exposed A549 cells showed that TiO2 NPs statistically induced DNA damage in only 100 µg/mL, which is the highest tested concentration in each particle sizes. **Conclusions:** Our results demonstrate that high concentrations of TiO2 NP's may induce DNA damage in A549 cells.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

NT 07- PRELIMINARY TOXICOLOGICAL ASSESSMENT OF THE COPOLYMER CHONDROITIN SULFATE-CO-N-ISOPROPYLACRYLAMIDE AS DRUGS CARRIER

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Introduction: A variety of polymers and/or copolymers have been evaluated by the pharmaceutical industry for drugs encapsulation, especially the ones which associate synthetic and natural polymers, due to their high stability, flexibility for chemical modification and specific biodegradability and because they promote controlled and precise release of the drug, reducing its toxicity and the administered dose. The chondroitin sulfate-co-N-isopropylacrylamide (CSM + NIPAAm) is a copolymer proposed for this purpose, from a synthetic polymer reaction, Poly N-isopropylacrylamide with thermosensitive characteristics with a natural Chondroitin Sulfate (CS), with bioadhesive characteristics. Thus, the copolymerization may be able to add these properties and to improve its use as a vehicle for controlled-release. **Objectives**: The aim of this work was to assess the toxicity of the copolymer CSM + NIPAAm 5% through both brine shrimp (*Artemia salina*) bioassay and cytotoxicity assay using PC-12 cells. **Material and Methods**: The copolymer CSM + NIPAAm 5% (provided by the EMBRAPA Amazônia Oriental, Belém – PA) was prepared in vials at different concentrations (1,000; 750; 500; 250;100 and 50 µg/mL) for the brine shrimp lethality bioassay. Survival trials (24 and 48 h) was assessed by scoring the number of dead nauplii (LC_{50} - probit analysis). Cell viability assay (cytotoxicity), by the metiltetrazolium (MTT) method, was carried out on PC-12 cells in 96-well plates ($2.0x10^4$ cells/well) incubated at different concentrations (2,000; 1,000; 500; 250 and 100 µg/mL) with the copolymer during 24 and 48 h. Data were expressed as mean □ standard deviation of mean. ANOVA and Bonferonni *pos-hoc* test were used for statistical analysis. **Results and Discussion**: The results showed which the copolymer CSM + NIPAAm 5% was unable to cause death of the *A. saline* nauplii (both 24 and 48 h) up to tested concentrations. For the cytotoxicity assay, there was no statistically significant difference between the control and the all tested concentrations, corroborating with the results obtained from the brine shrimp lethality bioassay. **Conclusion**: The copolymer CSM + NIPAAm 5% was shown to be nontoxic on the models and evaluated concentrations.

NT 08- EVALUATION OF GRAPHENE OXIDE TOXICITY BY FET TEST IN THE PRESENCE OF HUMIC ACID

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Introduction: Sustainable development of nanotechnology requires the deep evaluation of the safety of its products. Despite nanotoxicological studies have been intensified in recent years, gaps remain in the methods used to assess the nanotechnology risks. These gaps are due to the complex nanomaterials behavior in the environment, especially in the presence of organic matter and depending on the nanomaterial characteristics. Studies indicate that humic acid present in the aquatic environment can increase the stability of nanomaterial dispersions and may change its toxicity to aquatic organisms. **Objective:** The aim of this study is to evaluate the influence of humic acid in the toxicity of graphene oxide (GO) utilizing Fish Embryo Toxicity Test (FET test). **Materials and Methods:** Zebrafish embryos (*Danio rerio*) were exposed during 96 h to GO (100, 10 or 1 $mg.L^{-1}$, Sigma Aldrich) with or without humic acid (HA, 20 $mg.L^{-1}$, Sigma Aldrich). Control groups exposed to water and HA were performed. At the end of the exposure period the larvae were measured and frozen at -20°C for subsequent evaluation of biochemical biomarker of oxidative stress (catalase and glutathione S-transferase activity). The stability of suspensions was evaluated through spectrophotometry and dynamic light scattering. **Results and Discussion:** GO agglomerated and precipitated quickly in reconstituted water. The presence of HA in the medium stabilized the GO suspension similarly to that ocurred with GO in ultrapure water. There was no difference between groups related to the occurrence of embryo malformation, mortality or total length of the larvae. The parameters of sublethal effects will be further analyzed. **Conclusion:** GO did not show acute toxicity to zebrafish embryo and the presence of HA did not change acute GO effects. Nevertheless, sublethal effects must be evaluated to ensure GO safety.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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NT 09- GENOTOXICITY ASSESSMENT OF TITANIUM DIOXIDE NANOPARTICLES USING DIFFERENT PROCEDURES OF *ALLIUM CEPA* TEST

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Introduction: The increasing use of nanoparticles (NPs), such as titanium dioxide (TiO_2) NPs, in marketed products can lead to their release into the environment and their effects to ecosystem health are becoming a great of concern. Accurate prediction of NPs' toxicity should take into account their bioavailability to test systems. NPs' bioavailability can be impact, among others, by their aggregation to the materials used during experimental procedure, which prevent NPs' internalization into the cell, resulting in false negative outcomes. The *Allium cepa* test (phytotoxicity test) can be performed by different exposure procedures of test organism. **Objective**: This work compares different protocols of *A. cepa* test, aiming to minimize problems of NPs' bioavailability, which could interfere in their genotoxicity prediction. **Materials and Methods:** *A. cepa* was exposed to ultrapure water (negative control – NC); methyl methanesulfonate (MMS) at 10 mg/L (positive control); TiO_2 NPs at 1 mg/L (Zeta-potential: -4.92 mV) by three exposure procedures: (1) seeds germinated at polystyrene Petri dishes covered with filter paper; (2) seeds germinated at polystyrene Petri dishes covered with nylon net; (3) bulbs disposed in polystyrene recipients. TiO_2 NPs' solution was replaced every 24 h for a fresh one. The 2-cm long roots were fixed and used in the microscopic analyses. The results of each procedure were statistically (Mann-Whitney) compared according to mitotic index (MI), chromosome aberrations (CA) and micronuclei (MN) of treatments with negative control. Kruskal-Wallis statistical analysis was carried out to compare the different procedures. **Results and Discussion**: Significant increase of all endpoint analyzed (MI, CA, MN) were observed for TiO_2 NPs using seeds. Similar results were observed to bulbs' experiment, except for MI, which did not show significant difference related to NC. The comparison among the different procedures of *A. cepa* test shown that there was no difference between the two protocols using seeds, suggesting any interference of these procedures in NPs' bioavailability. On the other hand, significant difference was observed between the procedures with seeds and bulbs. Such fact may be related to sensitivity of seeds and bulbs instead of NPs' bioavailability, since, as seeds, bulbs' results also predicted the genotoxicity of TiO_2 NPs, but the CA and MN frequencies were lower than the ones obtained in seeds' protocol. **Conclusions:** All *A. cepa* test procedure was able to detect the genotoxic effects of TiO_2 NPs, being the protocols with seeds more sensitive than the one with bulbs. Furthermore, our data suggest that TiO_2 NPs can induce deleterious effects to exposed organism, such as the genotoxicity for *A. cepa* herein observed.

NT 10- GENOTOXICITY ASSESSMENT OF TITANIUM DIOXIDE NANOPARTICLES USING DIFFERENT PROCEDURES OF *ALLIUM CEPA* TEST

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Introduction: The increasing use of nanoparticles (NPs), such as titanium dioxide (TiO_2) NPs, in marketed products can lead to their release into the environment and their effects to ecosystem health are becoming a great of concern. Accurate prediction of NPs' toxicity should take into account their bioavailability to test systems. NPs' bioavailability can be impact, among others, by their aggregation to the materials used during experimental procedure, which prevent NPs' internalization into the cell, resulting in false negative outcomes. The *Allium cepa* test (phytotoxicity test) can be performed by different exposure procedures of test organism. **Objective**: This work compares different protocols of *A. cepa* test, aiming to minimize problems of NPs' bioavailability, which could interfere in their genotoxicity prediction. **Materials and Methods:** *A. cepa* was exposed to ultrapure water (negative control – NC); methyl methanesulfonate (MMS) at 10 mg/L (positive control); TiO_2 NPs at 1 mg/L (Zeta-potential: -4.92 mV) by three exposure procedures: (1) seeds germinated at polystyrene Petri dishes covered with filter paper; (2) seeds germinated at polystyrene Petri dishes covered with nylon net; (3) bulbs disposed in polystyrene recipients. TiO_2 NPs' solution was replaced every 24 h for a fresh one. The 2-cm long roots were fixed and used in the microscopic analyses. The results of each procedure were statistically (Mann-Whitney) compared according to mitotic index (MI), chromosome aberrations (CA) and micronuclei (MN) of treatments with negative control. Kruskal-Wallis statistical analysis was carried out to compare the different procedures. **Results and Discussion**: Significant increase of all endpoint analyzed (MI, CA, MN) were observed for TiO_2 NPs using seeds. Similar results were observed to bulbs' experiment, except for MI, which did not show significant difference related to NC. The comparison among the different procedures of *A. cepa* test shown that there was no difference between the two protocols using seeds, suggesting any interference of these procedures in NPs' bioavailability. On the other hand, significant difference was observed between the procedures with seeds and bulbs. Such fact may be related to sensitivity of seeds and bulbs instead of NPs' bioavailability, since, as seeds, bulbs' results also predicted the genotoxicity of TiO_2 NPs, but the CA and MN frequencies were lower than the ones obtained in seeds' protocol. **Conclusions:** All *A. cepa* test procedure was able to detect the genotoxic effects of TiO_2 NPs, being the protocols with seeds more sensitive than the one with bulbs. Furthermore, our data suggest that TiO_2 NPs can induce deleterious effects to exposed organism, such as the genotoxicity for *A. cepa* herein observed.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

NT 11- SKIN PERMEABILITY AND CYTOTOXICITY OF TOPOTECAN-LOADED LIPID NANOPARTICLES

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Introduction: Topotecan (TPT) is an important anticancer drug used in the treatment of ovarian, lung, and breast cancer. Cytotoxic activity of TPT in melanoma model has not been demonstrated since this drug undergoes rapid hydrolysis at neutral or alkaline pH. Thus, the encapsulation of TPT in lipid nanoparticles (NP) could increase drug stability, skin permeation and cytotoxicity activity, enabling local treatment of skin cancer. **Objective:** We investigated the potential efficacy of TPT-loaded lipid nanoparticles (TPT-NP) to increase drug permeation through pig ear skin and on its cytotoxicity effect on B16-F10 and 3T3 cell lines. **Material and methods:** NP were produced by microemulsion technique with stearic acid, oleic acid, soy lecithin and sodium tarodeoxicholate. TPT-NP had mean diameter of 119.14 ± 25.5 nm, polydispersity index of 0.191 ± 0.009 and drug entrapment efficiency of 96.33 ± 1.56%. Drug skin permeation studies were conducted in Franz diffusion cells and pig ear skin. After 24h, the stratum corneum (EC) was removed from the remaining skin (RS) by tape stripping technique. TPT quantitation was performed by high performance liquid chromatography method. B16-F10 murine melanoma and Balb/3T3 embryonic fibroblasts cell lines were grown in monolayer culture, trypsinized, added ($3x10^4$ cells/well) to culture microplates of 96-well. The cells were exposed to TPT, NP, or TPT-NP for 24 h at 37°C. The viability of the cells was measured using the colorimetric MTT and the inhibitory concentration of 50% (IC50) was determined. **Results and discussion:** The TPT-NP increased TPT permeation in both skin layers (EC and RS) when compared to the unloaded drug. The total amount of TPT accumulated in the skin after topical application of TPT-NP was approximately 29 µg/mL, significantly higher that IC50 found for B16-F10 (7.36 µg/mL) and significantly lower than the IC50 found for 3T3 (59.99 µg/mL). The cytotoxic activity of TPT-NP in 3T3 cells was altered to 2.16 µg/mL while for B16-F10 was to 0.84 µg/mL. NP did not show significantly cytotoxicity in the tested conditions. **Conclusion:** Our data suggest that NP significantly increased TPT skin permeation, improved cellular uptake and enhanced cytotoxicty using lower doses of TPT (IC50 of TPT-NP is lower than IC50 of TPT) for melanoma and non- cancerous cells. Therefore, TPT-NP may be an alternative therapy for melanoma skin cancer.

Financial Support: FAPEG, CAPES and CNPq.

NT 12- TOXICITY EVALUATION OF THE NOVEL PHOTOPROTECTIVE COMPOUND LQFM048 SYNTHESIZED THROUGH GREEN CHEMISTRY APPROACH

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Introduction: The new heterocyclic derivative LQFM048 (2,4,6-tris ((E)-ethyl 2-cyano-3-(4-hydroxy-3-methoxyphenyl)acrylate)-1,3,5-triazine) was originally designed through molecular hybridization strategy from Uvinul® T 150 and (E)-ethyl 2-cyano-3-(4hydroxy-3-methoxyphenyl)acrylate sunscreens, using green chemistry approach. This compound presented global yields of 78%, interesting redox potential and thermal/UVA stability. Since LQFM048 showed an increment of 64% in the photoprotection in relation to ethylhexyl metoxycinamate standard sunscreen, this compound has been considered a promising candidate to a novel photoprotective. **Objective:** To evaluate the safety of the LQFM048 using predominantly alternative methods. **Materials and Methods:** Eye irritation was evaluated by Short Time Exposure (STE), Bovine Corneal Opacity and Permeability (BCOP), Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) and hemolytic assay was performed using mice erythrocytes. Skin toxicity was investigated by MTT assay and IL-18 secretion in HaCaT keratinocytes, and Local Lymph Node Assay (LLNA):BrdU-ELISA. The i*n vitro* 3T3 neutral red uptake phototoxicity assay and micronucleus assay were also carried out. **Results and Discussion:** In eye irritation assessment, LQFM048 was non-cytotoxic in STE test and did not promote changes in the corneal permeability, opacity and histology. Additionally, presence of hemorrhage, vessel lysis and/or coagulation was also not detected in HET-CAM assay as well as non-hemolytic profile in erythrocytes. In skin toxicity evaluation, LQFM048 presented non-phototoxic in 3T3 cells, non-cytotoxic and did not change IL-18 secretion response in keratinocytes. In LLNA:BrdU-ELISA, hexyl cinnamic aldehyde and eugenol positive controls showed a stimulation index (SI) of 2.4 and 1.9, respectively, being classified as sensitizers. By the other hand, LQFM048 was considered as non-sensitizer (SI=0.7). Additionally, no clinical signs were observed in the mice. Moreover, LQFM048 did not show potential mutagenic. **Conclusion:** The new photoprotective compound LQFM048 showed safe for endpoints investigated and its use in cosmetic and pharmaceutical products with sunscreen property is promising. Moreover, the multiparametric platform obtained here seems interesting in the toxicity evaluation of new compounds.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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ANALYTICAL TOXICOLOGY

A | R | T
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AN 01- DESCRIPTION PROFILE OF EMERGENCY TOXICOLOGICAL ANALYSIS IN A TOXICOLOGY LABORATORY IN SOUTHERN BRAZIL

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Introduction: In attendance of acute poisoning, the toxicological analyzes should be available to identify or confirm suspected substance, and to determine the dose involved. To this purpose, the Toxicology Laboratory (TL) must have analytical resources to qualitative or quantitative fast analysis. **Objective**: Describe the profile of the demand for emergency toxicological analyzes and the time to release the results in a TL located at the University Hospital in Florianopolis-SC. **Methods**: Retrospective descriptive study based on the requests of toxicological analyzes and biological samples records sent to the TL in the first 10 months of official operation. **Results and Discussion**: In this period, the TL conducted 422 analyzes related to 415 patients through the validated, quantitative and qualitative techniques. Qualitative analysis were performed by immunochromatography (Drug Abuse Screening) and colorimetry (Paraquat).They were the most performed test (284/422) identifying 13 different substances. Of all qualitative analysis, 62.7% were "Detected". The drug abuse screening test represented 64.7% of total analyzes (273/422) and identified 12 substances per test, generating 3.276 results of individual drugs. The benzodiazepine group were the most frequently detected (80/3276). Among drugs of abuse, cocaine detection was predominant (50/3276), followed by marijuana (40/3276).The paraquat research was unless qualitative analysis requested (3.9%), but was detected in 45.4% of cases resulting in death in 60% of confirmed poisoning. Quantitative analyzes were performed by automatized method (Dimension®RxL - Siemens,Germany) and spectrophotometry. Among these analyzes, the plasma determination of acetaminophen was the most requested (54.3%). In 82.6% of poisoning by this agent was marked low risk for hepatotoxicity, in accordance with the Rumack-Matthew nomogram. The determination of the butyrylcholinesterase activity represented 14.3% of the requests and 46.6% of tests had results below the reference values, indicating the possible poisoning by cholinesterase inhibitors. The measurement of the percentual of methemoglobin was the quantitative analysis least performed (3/138). Thus, 29.7% of the total quantitative analyzes confirmed a possible acute intoxication by the agents studied.The estimated mean time for release of the results was 67 min (±44). **Conclusion**: There was a predominance of qualitative techniques and the drug abuse screening test was the most requested analysis. Taking together all the analyzes performed, 50.7% showed confirmatory results of exposure or acute poisoning and it helped in the diagnosis and medical management. Besides, the release time of the results was satisfactory, being in accordance with the deadlines for emergency analyzes.

AN 02- IMPLEMENTATION OF A TOXICOLOGY LABORATORY IN A UNIVERSITY HOSPITAL

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Introduction: The implementation of a toxicology laboratory allows to systematize and standardize the analyses already made bringing improvement and promoting quality for a better treatment of the patients acutely intoxicated. Other advantages are the economy in hospitalization, the reduced period of observation and the period of hospitalizations in the intensive treatment unities as well as the appropriate intervention with the use of antidotes and an adequate closure of the cases. **Objective:** Systematize and optimize the performance of emergency exams in a toxicology laboratory in a university hospital. **Materials and Methods:** It was performed a validation of the methodologies already executed, considering the parameters: linearity, intra and inter test precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) to plasma determination of acetaminophen by spectrophotometry, and the LOD to the paraquat identification in urine samples by colorimetry. The presentation of the analytical procedures, materials, instrumentation, needed inputs and the correct interpretation was done through the elaboration of Standard Operational Procedures (SOPs) to all the exams performed, including the two validated and also the screening of the abuse drugs performed by immunochromatographic method. It was elaborated also an orientation manual to the gathering, transport and storing biological samples. **Results and Discussion:** The method for the determination of acetaminophen demonstrated to be linear between 6,25 to 200 $\mu g.mL^{-1}$, producing LOD of 3,08 $\mu g.mL^{-1}$ and LOQ of 6,25 $\mu g.mL^{-1}$. Both test precision produced adequate values, with variation coefficients lower than 20% to the LOQ and lower than 15% to the other tested concentrations. Regarding the accuracy, the method didn't present any deviation exceeding ±15%. Thus, the validation of the methodology referred to demonstrate that the method is linear, sensitive, accurate and exact, thus certifying the reliability of the results generated during the application of it. It was determined that the LOD of the identification method of paraquat is 1,56 $\mu g.mL^{-1}$, corroborating the literature information. It was prepared protocols to the exams performed in the laboratory, containing the optimized descriptions to each procedure, allowing standardization to the techniques and unifying the results. **Conclusion:** The upgrading performed was fundamental in the sense of implementing processes and defined protocols, providing ways for this to occur in a satisfactory manner, as well as allowing optimization of the workflow, ensuring the quality of the analysis performed.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

AN 03- SIMULTANEOUS SCREENING OF ACETAMINOPHEN, DEXTROMETHORPHAN, ETHYL GLUCURONIDE, IBUPROFEN, SALICYLATE AND OTHER PRESCRIPTION DRUGS INCLUDING ANTIDEPRESSANTS ON A BIOCHIP PLATFORM

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Introduction: Prescription drugs are safe and effective when used properly but can be also harmful and addictive when abused, causing deaths in cases of overdose. Acetaminophen and salicylates, because of their prevalence in numerous over the counter preparations, result in cases of serious toxicity as well as fatalities. Cyclic antidepressants have a high case fatality rate. Dextromethorphan, a common ingredient in cough suppressants, is an opioid derivative with abuse potential. Ethyl glucuronide is a marker to test alcohol consumption. Fatal overdoses associated with ibuprofen have been reported. Toxicological analysis depends upon screening and confirmatory tests. Biochip array technology allows the screening of multiple drugs from a single sample leading to an increase in test results output. **Objective:** This study reports the analytical evaluation of a biochip array for the simultaneous detection of acetaminophen, dextromethorphan, escitalopram, ethyl glucuronide, fluoxetine, haloperidol, ibuprofen, methylphenidate, salicylate, sertraline, tramadol, trazodone and tricyclic antidepressants (TCAs) in urine and blood samples. **Materials and methods:** Matrix dedicated biochip array kits were used for drug determination in urine and blood. Competitive chemiluminescent immunoassays arrayed on a biochip surface were employed and applied to the Evidence analyser, which incorporates dedicated software to process and archive the multiple data generated. **Results:** Acetaminophen, dextromethorphan, escitalopram, ethyl glucuronide, fluoxetine, haloperidol, ibuprofen, methylphenidate, salicylate, sertraline tramadol, trazodone and TCAs were detected simultaneously with limits of detection ranging from 0.10 ng/mL (tramadol) to 17.36 µg/mL (ibuprofen) in neat urine and from 0.04 ng/mL (tramadol) to 6.42 µg/mL (ibuprofen) in neat blood sample. The assay for TCAs was standardised to nortriptyline but other TCAs were also detected with cross-reactivity (%) ranging from 22.1 (norclomipramine HCl) to 1127 (imipramine N oxide) in urine and from 24.3 (chlorpromazine) to 1127 (imipramine N oxide) in blood. The intra-assay and inter-assay precision (n=20) of the immunoassays for 3 different concentration levels, expressed as CV (%), was ≤17% in both matrices. **Conclusion**: Biochip array technology is therefore applicable to the matrix dedicated multi-analytical determination of drugs to maximise screening in test settings.

AN 04- DEVELOPMENT AND VALIDATION OF A BIONALYTICAL METHOD FOR QUANTIFICATION OF DASATINIB AND NILOTINIB AND ITS APPLICATION TO THERAPEUTIC DRUG MONITORING IN CHRONIC MYELOID LEUKEMIA

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Introduction: Chronic myeloid leukemia is a clonal chronic myeloproliferative disease, treated by means of tyrosine kinase inhibitors (imatinib, dasatinib and nilotinib). Dasatinib is a drug derived from piperazinyl and the nilotinib is a derivative of aminopyrimidin, being a similar and more powerful drug to imatinib[1,2]. To determine drugs concentration in human plasma, ANVISA recommends bioanalytical methods validated to generate reliable and interpretable information about the samples. Validation is a continuous process that starting in the analytical strategy planning and keeps going throughout their development[3].Validation must ensure applicable and reliable results in the laboratory routine. It is required that laboratories validate their methodologies according to the following parameters: selectivity, residual effect, matrix effect, linearity, accuracy, precision and stability[4]. **Objective:** To develop and validate a bioanalytical method to measurement dasatinib and nilotinib in human plasma. **Materials and methods:** Chromatography was performed HPLC-PDA Shimadzu Prominence model 20ATand C18 ACE® column (150 x 4, 6 mm; 5 µm) at 30 ºC was used. Mobile phase was acetonitrile and triethylamine 1 % pH 10,5 (40:60, v/v), flow 1 mL/min and injection volume 20 µL. Wavelength was 265nm for nilotinib and the internal standard 5-(4-methylphenyl)-5-phenylhydantoin (MPPH) and 323nm for dasatinib. Preparation samples was performed by protein precipitation with methanol and after liquid-liquid extraction with ethyl acetate. Protocol was approved both local and national ethics committee. **Results and discussion:** Total chromatographic run time was 15 minutes (MPPH = 2.35 min; dasatinib = 3.67 min and nilotinib = 9.15 min). Stabilities (short- and long-term, frozen cycles and auto sampler), quality controls and matrix effect showed coefficient of variation (CV %) lower than 15 % and limit of quantification (LOQ) lower than 20 %. Therefore, method was applied in the analyses of two patients, for nilotinib (P1 = 1.70 µg/mL and P2 = 1.09 µg/mL) and dasatinib (P3 < LOQ and P4 < LOQ). **Conclusion:** The validated method was selective, precise, accurate and linear, according parameters required by ANVISA and it may be include in a clinical routine monitoring of patients with chronic myeloid leukemia.

References:

1. Bollman P.W & Giglio A.Del. Einstein, **9** (2): 236 (2011).
2. Lopes NR, Abreu MTCL, *Rev Bras. Hematol Hemoter*, **31**(6): 449 (2009).
3. Ribani M et al. *Quim. Nova,* **27**(5): 771 (2004).
4. BRASIL. Agência Nacional de Vigilância Sanitária - ANVISA. RDC n◦27 de 17 de maio de 2012.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 05- DETERMINATION OF COCAINE, COCAETHYLENE AND NORCOCAINE IN HUMAN BREAST MILK SAMPLES USING LIQUID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Introduction: It is well known that some psychoactive substances can be excreted into human breast milk. However, information about the amount of substances present in this matrix is limited, as well their possible effects in the infant during nursing period. The quantification of drugs in breast milk could allow a better evaluation of this route of exposure in producing immediate and delayed adverse effects in the development. **Objectives:** The aim of the present study was to quantify the active compounds: cocaine (COC), cocaethylene (CE) and norcocaine (NCOC) in human breast milk samples. The analyses were performed by using liquid phase microextraction (LPME) and gas chromatography mass spectrometry (GC-MS). **Material and Methods:** Twenty (20) women aging 18 to 40 y.o. who gave birth in the maternity of the Hospital Municipal Dr Arthur Ribeiro de Saboya, accepted to participate of the study and answered a questionnaire about drug use. Breast milk samples were collected between 24-48 hours after delivery. For analytes extraction, 0.5 mL breast milk sample was diluted with 0.5 mL of borate buffer (pH 9.0) and submitted to a LPME technique based in the use of a polypropylene hollow fiber immersed in *n*-octanol. The extract was derivatized at 60ºC for 30 minutes with BSTFA-1%TMCS, and 1 µL of the final solution was injected into the GC-MS. This method was previously fully validated. Deuterated internal standards (COC-d3, CE-d3, and NCOC-d3) were used in all samples and calibration curves (12, 50, 100, and 250 ng/mL) were built for quantification. **Results and Discussion:** The calibration curves were suitable for the quantification of the analytes. Twenty breast milk samples were analyzed by GC-MS, from which five women have reported cocaine consumption through the questionnaire. Only one sample was confirmed COC positive (138 ng/mL). Another one presented COC concentration near the limit of detection of the method (5 ng/mL), and it could not be quantified. The remaining samples were negative for COC, CE and NCOC. **Conclusions:** The LPME/GC-MS method has shown to be a reliable alternative for the determination of cocaine in human breast milk, and to characterize the importance of this route of exposure to the infant. Although the results showed that the transfer of cocaine to breast milk is possible, further investigation in positive samples is needed to evidence the severity of neonate poisoning.

AN 06- MULTI-ANALYTICAL METHOD VALIDATION FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF SOLVENTS OF ABUSE IN ORAL FLUID BY HS-GC/MS

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Introduction: The use of oral fluid as a biological matrix to monitor the use of drugs of abuse is a global trend because it presents several advantages and good correlation to the blood level. **Objective:** The present work aimed to develop and validate an analytical method for quantification and detection of solvents used as inhalants of abuse in oral fluid (OF), using Quantisal™ as collector device by headspace and gas chromatography coupled with a mass detector (HS-GC/MS). **Materials and Methods:** . For the simultaneous quantification of ethanol, diethyl ether, dichloromethane, chloroform, ethyl acetate, and n-butanol, the parameters of linearity, precision, accuracy, residual effect, matrix effect, limit of detection and quantification, and stability were assessed. To perform the analyses, headspace temperature was maintained at 85 °C for 5 min of incubation. Chromatographic separation was performed with a ZB-BAC1 column (30 m x 0.32 mm x 1.8 µm), and the total time of analysis was 11.8 min.The validation was determined according to the RDC 27/2012 (ANVISA). **Results and discussion:** The method showed good linearity with a correlation coefficient higher than 0.99 for all solvents. The limits of detection ranged from 0.05 to 5 mg/L, while the lower limits of quantification ranged from 2.5 to 12.5 mg/L. Accuracy, precision, matrix effect, and residual effect presented satisfactory results with the coefficient of variation (CV) below 15%, and relative standard deviation (RSD) ± 15%, meeting the criteria accepted for the validation of bioanalytical methods. The method showed good selectivity considering that, for solvents coeluting at the same retention time, resolution was performed by the mass detector. **Conclusion:** The method developed proved to be adequate when applied in OF samples from users of drugs, it is also a quick, practical, and sensitive method that may be used to monitor the abuse of inhalants in routine forensic analyses.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

AN 07- DETERMINATION OF COCAINE, COCAETHYLENE AND NORCOCAINE IN HUMAN BREAST MILK SAMPLES USING LIQUID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

[1]SILVEIRA, G.O.; [1]BELITSKY, I.T.; [2]LODDI, S.; [2]OLIVEIRA, C.D.R.; [2]ZUCOLOTO, A.D.; [2]FRUCHTENGARTEN, L.V.G; [1]YONAMINE, M.

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Introduction: It is well known that some psychoactive substances can be excreted into human breast milk. However, information about the amount of substances present in this matrix is limited, as well their possible effects in the infant during nursing period. The quantification of drugs in breast milk could allow a better evaluation of this route of exposure in producing immediate and delayed adverse effects in the development. **Objectives:** The aim of the present study was to quantify the active compounds: cocaine (COC), cocaethylene (CE) and norcocaine (NCOC) in human breast milk samples. The analyses were performed by using liquid phase microextraction (LPME) and gas chromatography mass spectrometry (GC-MS). **Material and Methods:** Twenty (20) women aging 18 to 40 y.o. who gave birth in the maternity of the Hospital Municipal Dr Arthur Ribeiro de Saboya, accepted to participate of the study and answered a questionnaire about drug use. Breast milk samples were collected between 24-48 hours after delivery. For analytes extraction, 0.5 mL breast milk sample was diluted with 0.5 mL of borate buffer (pH 9.0) and submitted to a LPME technique based in the use of a polypropylene hollow fiber immersed in *n*-octanol. The extract was derivatized at 60ºC for 30 minutes with BSTFA-1%TMCS, and 1 µL of the final solution was injected into the GC-MS. This method was previously fully validated. Deuterated internal standards (COC-d3, CE-d3, and NCOC-d3) were used in all samples and calibration curves (12, 50, 100, and 250 ng/mL) were built for quantification. **Results and Discussion:** The calibration curves were suitable for the quantification of the analytes. Twenty breast milk samples were analyzed by GC-MS, from which five women have reported cocaine consumption through the questionnaire. Only one sample was confirmed COC positive (138 ng/mL). Another one presented COC concentration near the limit of detection of the method (5 ng/mL), and it could not be quantified. The remaining samples were negative for COC, CE and NCOC. **Conclusions:** The LPME/GC-MS method has shown to be a reliable alternative for the determination of cocaine in human breast milk, and to characterize the importance of this route of exposure to the infant. Although the results showed that the transfer of cocaine to breast milk is possible, further investigation in positive samples is needed to evidence the severity of neonate poisoning.

AN 08- VALIDATION OF A METHODOLOGY FOR QUANTIFICATION OF MERCURY IN BIOLOGICAL TISSUES BY COLD VAPOR ATOMIC ABSORPTION SPECTROMETRY (CV-AAS)

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INTRODUCTION: Mercury (Hg) is a chemical element found in nature in different forms, which can be toxic to human being. The distribution of this metal is widely to various tissues, including, liver, kidney, muscle and brain. The Hg intoxication can cause severe and irreversible problems, comprising diseases in respiratory, digestive and nervous systems, which can even cause death. In this context, an analytical methodology to quantify Hg in biological tissues becomes an important tool for the research. **OBJECTIVES:** To validate a method for Hg's quantification in tissues by CV-AAS. **MATERIALS AND METHODS:** To CV technique were used HCl 18% as a carrier agent and $NaBH_4$ 1% with 0.25% of NaOH as reducing agent (Quim.Nova 33:1285, 2010). Mice's brains were prepared by acid digestion. The validation was carried out following different guides including ANVISA (2003; 2012).The linearity was analyzed with three calibration curves with six levels, and evaluated by the correlation coefficient (r) and the Relative Standard Deviation (RSD) of replicates. The limits of detection (LoD) --and quantification (LoQ) were determined by mathematical method. Precision was analyzed with three spiked samples at levels 1.5, 3.5 and 5 µg/L and analyzed in triplicate at three different days. The RSDs of replicate were evaluated. Recovery studies were performed with spiked samples in the same levels that precision. To analyze the matrix effect, the slopes (*m*) of water's and matrix's curves were compared by statistical Student's t-tests, with a confidence interval of 95%. The experiments with mice's brains were approved by the Ethics Committee for Animal Research of the Federal University of Santa Catarina (n° PP00745). **RESULTS AND DISCUSSION:** The r obtained from calibrations curves was 0.9994, what represents good linearity at the range of 0.5 to 6.0 µg/L. The RSDs found were less than 15% in the lowest level and less than 10% in the others levels. The LoD and LoQ of the method were 0.13 and 0.43µg/L, respectively. These results illustrate a good sensitivity of the method. The RSDs' values found for within- and between-day precision were below 5% and 10%, respectively, demonstrating a good reproducibility. The recovery results were between 80-90% for the three levels studied. These results are consistent with those found on literature. The t-test's results showed no statistical differences between *m* from matrix's and water's curves, evidencing that there isn't any matrix effect. **CONCLUSION:** The evaluated parameters for analytical method validation exhibited the acceptable values, showing its suitability for quantification of Hg in biological tissues. The method validation assurance the quality of the data and this process is vital issue for application by researchers.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 09- FAST PROCEDURE FOR DETERMINATION OF GAMA-HYDROXYBUTIRATE (GHB) IN URINE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Introduction: Gamma hydroxybutyrate (GHB) is a drug that causes effects such as hypnosis and anterograde amnesia. GHB is used to promote sexual abuse and rapes and its analysis is considered a forensic challenge because of rapid excretion, endogenous GHB production and other organic acids similarly to this drug excreted in the urine. **Objective:** Rapid determination of GHB in urine samples by liquid-liquid extraction (LLE) using gas chromatography-mass spectrometry (GC-MS) analysis. **Materials and Methods:** 250 µL of urine placed in a microtube and added 10 µL (250 µg/mL) of GHB-D_6 as internal standard. Sample is acidified with a solution of 3 mol/L HCl to pH 2 with addition of 500 µL of ethyl acetate. Microtube is closed and shaken for 10 seconds at 2400 rpm in a vortex. After stirring, the material is brought into microfuge (1 min at 5000 rpm). 350 µL of organic phase are taken and transferred to vials containing anhydrous sodium sulfate. The supernatant from this step is placed in vial for drying at 40°C under N_2 flow. After drying, 35 µL of BSTFA are added with shaking and incubation for 10 min at 70°C. Subsequently, samples are cooled to room temperature and 2 µL are injected into the GC-MS in selected ion monitoring mode. Ions monitored were *m/z* 233, 204 and 117 and *m/z* 239, 210 and 123 for GHB and GHB-D_6 (underlined ions are used for quantification). **Results and Discussion:** BSTFA is considered the best choice for derivatization due to its low cost and handling facilities. Urine sample at pH 2 extracted with ethyl acetate in the absence of NaCl were chosen conditions. Stirring frequency does not influence the extraction of the analyte. A quick procedure (10 seconds) was chosen for method validation for achieving the concentration limits proposed for GHB in sexual assaults (10 µg/mL). The methodology was validated according to United Nations of Office on Drugs and Crime (2009) and showed linearity (0.5-20 µg/mL; $y = 0.1144X - 0.1247$; $R_2 = 0.9932$). It was not observed heteroscedasticity phenomenon. No chromatographic interference was observed after 13 different substances tested. Other parameters evaluated were limit of detection and quantification (0.25 µg/mL and 0.5 µg/mL); recovery (20-23.9%); intra-day precision (8.6-13.7%); inter-day precision (9.4-11.6%); accuracy (93.2-111.9 µg/mL); dilution integrity (10 times: 6% and 101.1% for precision and accuracy respectively). The proposed method is fast (less than 12 min) and allows the GHB determination in a working range compatible with lower concentrations proposed in other studies without changes. **Conclusion:** Procedure developed for the GHB determination in urine samples is simple and extremely fast, including lower concentrations suggested by other studies.

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AN 10- QUALITY CONTROL AND QUALITY SPECIFICATION FOR THE PLASMA DETERMINATION OF ACETAMINOPHEN BY SPECTROPHOTOMETRY

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Introduction: The plasma determination of acetaminophen by spectrophotometry is an analysis used in emergency services due to its low cost, simplicity and speed. However, in order to contribute for a diagnosis with liability, it is necessary a quality system for the institution. **Objectives:** To propose a standardization of the internal quality controls (IQCs) for the plasma determination of acetaminophen by spectrophotometric method. **Materials and Methods:** Four different concentrations for the IQCs were defined previously – 10, 70, 110 and 150 $\mu g.mL^{-1}$. Then it was performed 20 determinations of them to obtain mean, standard deviation (SD) and the relative standard deviation (%RSD). It was evaluated whether or not the dispersion obtained was compatible with the specifications of the Total Allowable Error (TAE) to the acetaminophen recommended by the Royal College of Pathologists of Australasia (RCPA): ± 3 $\mu g.mL^{-1}$ for the lower concentrations and ± 10 % for the higher concentrations than 30 $\mu g.mL^{-1}$. The comparison of the obtained results in the IQCs was carry out estimating the percentage value of the Total Error (TE). The acceptable limits of the IQCs considered the concentration range determined by ± 2 SD in relation to the obtained mean. **Results and Discussion:** Analyzing the determinations of the IQCs, it was obtained a TE equal to 4.39 $\mu g.mL^{-1}$ for the concentration lower than 30 $\mu g.mL^{-1}$ and for the concentrations above 30 $\mu g.mL^{-1}$ the TE was 6.33%. Thus, for the concentrations above 30 $\mu g.mL^{-1}$ the method comply with the quality specifications and the value inside of the maximum analytical limit error allowed for a result. However, to the concentration of 10 $\mu g.mL^{-1}$ it was not attained the acceptable limit, with the possibility of representing an unacceptable quality result and demonstrating a reduced performance in low concentrations. As an alternative, the validation proposal for the daily calibration curve for this method is that it is performed based in only one rule, rejecting it when the control measurement exceeds the interval of ± 2SD. The immediate analysis of the result considering the acceptable range is fundamental to avoid the release of erroneous results. **Conclusion:** The standardization of IQCs for the plasma determination of acetaminophen by spectrophotometry allows certifying the results liability generated during its application including the concentration range of these values that are more correct and reliable. As there is no availability of Proficiency Testing for this method, besides the IQC it would be interesting the implementation of an alternative external evaluation, like the interlaboratorial comparisons, in order to guarantee the emission of accurate and consistent results.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 11- VALIDATION METHOD FOR BLOOD DETERMINATION OF CHLORPYRIFOS, FENITROTHION AND METILPARATHION BY GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY

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Introduction: Cholinesterase inhibitors are the main class of insecticides involved in cases of poisoning and occupational exposure. The exposure control can be accomplished by measuring the inhibition of cholinesterase activity; however, there is a great difficulty in the interpretation of an isolated evaluation. In addition, the implementation of a pre-occupational activity is not always possible. The proposal of new methods and new biological indicators of occupational exposure is needed. **Objective:** Validate a method using Gas chromatography with mass spectrometry (GCMS) for blood determination of Chlorpyrifos (CLOR), Fenitrothion (FEN) and Metilparation (MP). **Materials and Methods:** The 3 analytes were added to a blood pool with EDTA, in 6 different concentrations. Phorate (FOR) was used as internal standard (IS). The samples of total blood (500µL) were extracted using 1.5 mL of toluene and chloroform (4:1, v/v). The extract was resuspended with Hexane (1mL) and 1µL of the samples were injected into a GCMS-QP2010 Ultra (Shimadzu, Japan), with Split Ratio 40. The chromatographic separation was performed on a capilar column RTx®-5MS (30 m x 0,25 mm x 0,25 µm), using Helium as carreator gas. The ions **109**, 263, 79 (MP); **125**, 227, 260 (FEN); **97**,197, 99 (CLOR) and **75**, 121, 97 (FOR) were monitored and used the SIM acquisition mode for identification and quantification of each analyte. The validation parameters analyzed were: Limit of Detection (LOD), Limit of Quantitation (LOQ) Linearity, Intra- and Inter-day Precision, Stability and Recovery, according to the RE 899/2003 of ANVISA. **Results and Discussion:** The method demonstrated a good linearity in the studied concentration ranges to each analyte (0,5 to 15 $\mu g.mL^{-1}$ for CLOR; 0,6 to 15 $\mu g.mL^{-1}$ for FEN; 0,7 to 15 $\mu g.mL^{-1}$ for MP), with correlation coefficient of (r)>0,99 in all the analyzed curves. It was presented a Relative Standard Deviation (RSD%) under 10% in the intra-day precision (7,73% for CLOR; 4,77% for FEN; 4,9% for MP) and 15% in the inter-day precision (11,87% for CLOR; 11,12% for FEN; 10,83% for MP), showing to be a precise and reliable method. It was also presented a good sensibility for the 3 tested analytes with LODs of 0,12 / 0,18 / 0,2 $\mu g.mL^{-1}$ and LOQs of 0,5 / 0,6 / 0,7$\mu g.mL^{-1}$ for the CLOR, FEN and MP, respectively. The recovery was effective, with averages from 77 to 96% (± 5,4). The samples were considered stable when kept in refrigeration for 3 weeks, presenting variations inferior to 15% when compared to the freshly prepared samples. **Conclusion:** The validation of the studied method was satisfactory, demonstrating that one can be used to determine the blood concentration of Chlorpyrifos, Fenitrothion and Metilparation by means of GCMS in the workers exposed to these agents.

AN 12- ESTIMATION OF THE MEASUREMENT UNCERTAINTY IN QUANTITATIVE DETERMINATION OF BLOOD ETHANOL

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Introduction: For toxicologists measure blood ethanol concentration it may be necessary to consider multiple specification limits depending on the case's particular circumstances and up until now there have been relatively few measurement uncertainty (MU) publications. Furthermore, MU has been increasingly required in analytical toxicology by quality management standards, such as ISO 17025. It is also crucial to demonstrate reliability of analytical data and it is required as a validation parameter. Most laboratories take on decisions based on analytical data, meaning that having a small and reliable MU reflects the accuracy of the method used. **Aims:** To estimate MU in the analysis of quantitative determination of blood ethanol by headspace technique submitted to analysis through gas chromatography (HS/GC). **Methods:** Briefly, 1 mL of internal standard (n-propanol 0.6 g/L) and 0.5 mL of distilled water were added to 0.5 mL of whole blood. The sample was dispensed into 10 mL headspace vials which were appropriately sealed with butyl rubber septa and aluminum crimp seals. After sample heating (70 ºC for 30 minutes), a measured volume of headspace (500 µL) was injected in the GC and the volatiles separated on a capillary GC column with proprietary stationary phase followed by flame-ionization. Meth has been validated and the MU values were calculated following the Guide to the Expression of Uncertainty in Measurement. **Results:** The limit of detection (LoD) and quantification (LoQ) were of 0.005 and 0.1 g/L, respectively and the calibration curves were linear over the specified range (0.1 g/L to 5 g/L; r^2>0.99). The intra-day and inter-day precisions, in the lower concentration levels, have always been less than 20% considering relative standard deviation and accuracy values have been satisfactory (values above 93%). Once the method was validated it was applied to human blood samples collected at Vila Serena Drug and Alcohol Abuse Treatment Center and uncertainty values were calculated for these. In order of importance, the factors which appeared to be the most crucial ones for the calculation of method uncertainty were: analyte concentration, method precision, equipment uncertainty and sample volume. Combined uncertainty values averaged around 6.0%. **Conclusions**: This work has established the MU for the determination of ethanol in whole blood samples using HS/GC. The combined uncertainty was acceptable, supporting the successful application of the analytical method as it has shown to be both relevant and reliable.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 13- DEVELOPMENT CHROMATOGRAPHIC ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SALIVA USERS OF *CRACK*

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Introduction: Polycyclic Aromatic Hydrocarbons (PAHs) are a group of mutagenic and carcinogenic compounds with two or more condensed aromatic rings which are largely formed in incomplete combustion or pyrolysis of organic matter. In the act of smoking *crack*, these substances present in smoke are largely absorbed in the oral mucosa and in the lower airways. Therefore, the determination of the main carcinogenic PAHs in the saliva of crack users can contribute to the risk assessment of carcinogenesis and to the deterioration in the clinical picture of these individuals. **Objective**: Develop an analytical method for the simultaneous determination of the main carcinogenic PAHs (3,4-Benzo[a]pyrene, 3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene). **Materials and Methods**: The separation of the three compounds was carried out employing the High Performance Liquid Chromatography (HPLC) coupled with photodiode array detector, through a reverse phase system, using a C18 column (250cmx4,6nmx5um) and mixtures solvents (Methanol, Acetonitrile and Ultrapure Water). In order to reduce possible enlargements of peak were also evaluated acidic and basic mobile phase additives, as diethylamine and triethylamine. **Results and Discussion**: The chromatographic conditions for analysis were obtained by isocratic elution mode at temperature of 25°C and flow of 1mL/min. The estimated analysis time was about 15 minutes and the detection of compounds was performed in 270nm. To prove the most appropriate mobile phase reading of the analytes of interest, it was employed some solvents and additives. After optimization, the best analysis condition obtained was Acetonitrile:Ultrapure Water (65:35). The separation was satisfactory after adequate resolution of analytes, besides the number of theoretical plates (N), retention factor (k), and separation factor (α) coherent with the quantification of toxic agents in samples of human saliva to the application in analysis toxicological. The analyzed results of the chromatographic parameters for 7,12-dimethylbenz[a] anthracene were k=3,41; N=5.565,83; for the compound 3,4-Benzo[a]pyrene: k=2,94; N=7.988,40; and, finally, for the compound 3-methylcholanthrene: k=65,23; N=3.713,19. The α obtained from the compounds was in the range from 1,45 – 4,759 and the resolution between 2,53 – 3,21; which proved the effectiveness of the separation of the compounds. **Conclusion**: The chromatographic separation method proved to be appropriate for the simultaneous analysis of PAHs. After its development, the next step will be the collection of saliva samples from volunteers in internal CAPS AD Eulâmpio Cordeiro-Recife, performed by swab technique. The methodology will be validated in accordance with Resolution RE No. 27 of 2012 - ANVISA and employed in an evaluation study of the inducing potential of PAHs in the development of oral cancer in volunteers under clinical treatment for *crack* abuse.

AN 14- DEVELOPMENT OF ANALYTICAL METHOD FOR DETERMINATION OF COCAINE'S MAJOR ADULTERANTS IN HUMAN URINE

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Introduction: Cocaine is a stimulant of the central nervous system that features a strong ability to cause dependence. Often adulterants are added to this drug in order to mimic its action or minimize its adverse effects. When there are other pharmacologically active components in the drug composition, adverse reactions and other severe health problems can occur. This situation associated with the rising use of cocaine in Brazil could result in the increase of the number of users who seek health services presenting intoxication symptoms. Accordingly, there is a growing interest in developing methods for the determination of adulterants in biological samples of intoxicated cocaine users who are hospitalized. **Objectives:** To develop a novel method for the determination of the main adulterants of cocaine (caffeine, levamisole, lidocaine, phenacetin, diltiazem, and hydroxyzine) in human urine. **Methods:** The high-performance liquid chromatography coupled with a photodiode array detector (HPLC-DAD) and the dispersive liquid-liquid microextraction based on solidification of floating organic drop (DLLME-SFO) were used as analysis technique and as sample preparation technique, respectively. Propranolol was used as internal standard. The kind of extraction and disperser solvent were investigated for unifactorial design. The buffer pH, the volume of extraction and disperser solvent, and ionic strength were optimized for full factorial design. This study was approved by the ethics committee under the protocol 41943415.7.0000.5546. **Results and discussion:** The reversed-phase chromatographic separation was obtained with a column C18 extended (250x4.6mm; 5µm; 80Å) in gradient elution mode using acetonitrile-trifluoroacetic acid 0.026% (v,v) at 1 mL min^{-1} as mobile phase, at 25°C, and detection at 235nm. The analysis time was 25min. This condition had the best resolution factors (>1.15), retention factors (>0.68), number of plates (>2,094.9), and separation factors (>1.05) for all targets indicating a good separation. Under optimum conditions, human urine samples were alkalized with 0.5M sodium phosphate buffer (pH 12) and added to sodium chloride (20%m/v). Acetonitrile (150µL) and 1-dodecanol (30µL) were used as disperser and extraction solvent, respectively. **Conclusion:** This method was successfully developed and was shown to be a green alternative compared with other conventional extraction techniques. It will be validated and applied in urine of cocaine users. It is expected that this method will contribute to the speed and accuracy in the diagnosis of intoxication, the proper planning of therapeutic measures, as well as to the favorable prognostic.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 15- DETERMINATION OF TAFENOQUINE IN HUMAN PLASM BY DISPERSIVE-LIQUID-LIQUID MICROEXTRACTION (DLLME) COMBINED WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-ULTRAVIOLET DETECTION (HPLC-UV)

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Introduction: **Tafenoquine is an 8-aminoquinolone,** synthetic analogue of primaquine which is in fase III tests for the treatment of human malaria. It shows better antimalarial activity than primaquine in vitro and is usually well tolerated in patients. There are some methods for tafenoquine quantification in human plasm, but none uses Dispersive-Liquid-Liquid Microextraction (DLLME), an extraction technique that shows as mean advantages: miniaturization, low cost, high efficiency of extraction and preconcentration and high potential for routine application. **Objective: The aim of this study was to develop a method for quantification of tafenoquine in human plasm using DLLME combined with** high-performance liquid chromatography-ultraviolet detector (HPLC-UV).**Materials and Methods**: After protein precipitation, *tafenoquine was* extracted from plasma by DLLME using chloroform and acetonitrile as extraction solvents and dispersants, respectively. The mobile phase consisted of Methanol/Acetonitrile/Sodium acetate (10 mM, pH 6.7, 25^{o}C)/acetic acid (50:30:20:0.1 v/v/v) and a C18 column (15 x 4.6mm, 5mm) was used. The UV detection was performed at 262nm and 1 mL/min as flow rate. The following factors that influences the extraction efficiency were evaluated and optimized: dispersing solvent, solvent extractor, extractor solvent volume, pH and extraction time. Evaluations were made based on the areas of the chromatographic peaks obtained. **Results and Discussions:** During optimization, there were evaluated for extractor solvent chloroform, carbon tetrachloride, dichloroethane and tetrachlorethylene; and for dispersant solvent methanol, acetonitrile and acetone. Chloroform and acetonitrile showed higher recovery. It was also evaluated the volume of the solvent extractor (10, 20, and 30% of a solution containing solvent extractor and dispersant), with the volume of 30% showing higher recovery. For pH assessment was added to the extraction system 50, 100 and 200µl of 0.5M NaOH solution, and it was observed better extraction with the volume of 100 µL. Finally, the extraction times were also tested, and 1, 2 and 4 minutes were verified and the greater recovery were observed with 2 minutes. With the optimized method, the observed recovery was 35.48%. The upper and lower limits of quantification were 1500ng/ml and 50 ng/ml, respectively. **Conclusion**: This method showed satisfatory recovery and would be useful in routine analyses, specially for the simplicity, speed and low use of solvent of the DLLME technique.

AN 16- COMPARATIVE EVALUATION OF IMMUNOCHROMATOGRAPHY AND GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY FOR DETECTION OF COCAINE AND ITS METABOLITES IN URINE OF PATIENTS FROM DRUG MONITORING PROGRAMS

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Introduction: drug monitoring is one of the fields of analytical toxicology focusing in a clinical evaluation that requires rapid, sensitive and specific methodologies for the identification of drugs of abuse and its metabolites in human biological samples. Therefore, guaranteeing a high performance of these diagnostic tests implies in avoiding an early or a later release of patients under chemical dependency treatment, increasing treatment's cost. **Objective:** this study aimed to compare the triage analysis for drugs of abuse done by immunochromatography (IC) and a confirmatory analysis performed by gas chromatography/mass spectrometry (GC/MS). **Materials and methods:** 103 urine samples from patients under drug monitoring were analyzed by IC with posterior confirmation by GC/MS at the NAL/CIT/RS. For GC/MS analysis, Solid Phase Extraction (SPE) was adapted from Agilent's Methods Manual (2014). Samples were divided into two groups: positive for cocaine and metabolites with IC (n=50) and negative (totally negative or doubtful samples, those that present a weak band) with IC (n=53). This study was approved by the ethics committee of UFSCPA (number: 31797814.3.0000.5345). A 2x2 table was created to allow the calculations of the positive and negative predictive values (PPV and NPV), sensitivity and specificity comparing IC to GC/MS. **Results and discussion:** the PPV value indicated that 96% of all positive results with IC were truly positive; Nevertheless, the NPV value indicated that 69.81% of all negative results were truly negative. Sensitivity indicated that 75% of all true negative samples were detected with IC, however, 25% of the negative group (n=16) was composed by samples that presented a weak band (doubtful). This result indicates that all doubtful results should be confirmed by another methodology, considering that the cutoff levels for IC (150ng/mL) is higher than the ones for GC/MS (100ng/mL), and so the fact that patients under this treatment can present low concentrations of the drug. Specificity results indicate that IC detects correctly 94.27% of all positive cases. Considering that the Substance Abuse and Mental Health Services Administrations (SAMHSA) indicates that only positive results should be confirmed, findings of this study show that, despite the fact that IC presents a good specificity, the sensitivity can be affected with reduced concentrations that are presented in doubtful results, therefore, those results should be confirmed in the same methodology as the positive results. **Conclusion:** IC results showed that it is a specific and sensitive methodology when compared to GC/MS, however, special attention should be given to doubtful results in triage analysis. Therefore, a confirmatory analysis is indicated.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 17- FACTORIAL DESIGN TO STUDY STABILITY OF THE ETHANOL METABOLITES ETHYL SULFATE AND ETHYL GLUCURONIDE IN *POSTMORTEM* HUMOR VITREOUS (VH) AND WHOLE BLOOD (WB)

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Introduction: Ethyl sulfate (EtS) and Ethyl glucuronide (EtG) are minor ethanol metabolites used as specific markers to document recent alcohol consumption and provide legal proof of drinking.[1,2] **Objective**: The aim of this study was to investigate the stability of EtG and EtS in VH versus WB samples. The use 2^2X3^1 mixed factorial experiments performed. **Materials and methods**: To determine the volatile compounds using High Resolution Gas Chromatography (HRGC) with flame ionization detector and separation technique confined space. High performance liquid chromatography with detector resolution mass spectrometry and electrospray ionization was used to carry out the analysis of EtS metabolites and EtG. **Results and Discussion**: This study presents the results of a 2^2X3^1 mixed factorial experiment performed on three autopsied corpses in which was suspected alcohol consumption prior to death. The effects of collection tube, storage time and storage temperature on the stability of ethanol and the two biomarkers were studied under conditions of *postmortem* degradation in vitreous humor (VH) and whole blood (WB). In another 10 cases, there was no trace of ethanol, EtS or EtG in the specimens analyzed stored at room temperature (RT) after addition of 25 dg/L of ethanol, and no new formation of EtS and EtG was observed. VH and WB of the three corpses, with humor vitreous alcohol concentrations (VHAC) of 5.1, 13.5 and 40.9 dgL^{-1}, were stored for 4 weeks and analyzed periodically. **Conclusion**: EtS concentrations remained relatively constant when stored at RT for 4 weeks in tight vials. The variables showed different magnitude of effects on variations of EtS and EtG concentrations. EtS concentrations decreased 5% on average and EtG concentrations decreased 35% on average in 4 weeks storage at RT, but EtG was still detectable in all samples. *Postmortem* formation of EtS and EtG was not found in these *in vitro* experiments, which supports the hypothesis that EtS and EtG concentrations in vitreous humor prove alcohol consumption prior to death.

References
1. F. M. Wurst, G. E. Skipper, W Weinmann, *Addiction* 98 (Suppl 2):51–61(2003).
2. R.Z. Litten, A.M. Bradley, H.B Moss, *Alcohol Clin Exp Res* 34(6):955–967 (2010).

AN 18- DETERMINATION OF COCAINE AND BENZOYLECGONINE IN BREAST MILK BY MAGNETIC N-DOPED CARBON NANOTUBES AND GAS CHROMATOGRAPHIC-MASS SPECTROMETRY

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Introduction: The continued use of cocaine during pregnancy in recent years in Brazil, there has been growing concern regarding its effects on the fetuses and neonates. Breast milk is an unconventional array that has been used to evaluate the acute neonatal exposure to drugs due to its advantage facility of sample collection. Analyses of drugs in breast milk are essential to evaluate exposition, protecting infants of side effects and risks from maternal consumption of any legal or illegal drug. Besides, this information allows competent authorities making decision about mother–infant interactions. **Objective:** The of this study was to develop a new method for analysis of cocaine (COC) and benzoylecgonine (BE) in breast milk using magnetic carbon nanotubes (mCNT) and gas chromatography mass spectrometry (GC/MS). **Materials and Methods:** A factorial and Doehlert planning were used to optimize the method parameters. The variables selected for multivariate optimization with respective lower and higher levels were pH (4.00 and 8.00), mCNT mass (4.0 and 10.0 mg), adsorption and desorption time of analyte in nanotube (1 and 3 min).The experiments were carried out with breast milk free of analytes obtained by mixing the 5 donor milk (pool), in the concentration of 200 ng mL^{-1} of COC and BE. All statistical analyzes were performed using Statistica 8.0. **Results and Discussion:** Selected variables influence was evaluated by a fractional planning 2^{4-1} with central point, through eleven experiments. The importance of each variable was analyzed with p-value, the variables that were significant (p-value lower than 0.05) at a 95.0 % confidence level were pH and NTC mass at higher levels. Following, these variables were evaluated on several levels using Doehlert response surface obtained by the desirability function, through nine experiments. The other variables were held constant. Finaly, optimal experimental conditions obtained analyzing the response surface were 9.0 mg of mCNT mass in pH 8.00. **Conclusion:** Multivariate optimization has improved the extraction efficiency of COC and BE in breast milk. Within the experimental domain assessed mCNT mass and pH were the variables that influenced significantly the extraction of analytes.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 19- ALKALOIDS IN SPECIES OF *ERYTHROXYLUM*

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Introduction: The family Erythroxylaceae (Angiosperms) presents pantropical distribution and the alkaloids are the principal components these plans. The phytochemical and possibility variation phytochemical as the seasons in species the genus *Erythroxylum* in Brazil not´s very studied. The alkaloids presents are derivatives of ecgonine as cocaine, benzoylecgonine and methylecgonine. **Objective**: Evaluate the content in alkaloids derived from ecgonine species collected in South and northeast of Brazil during the summer and winter.**Materials and methods**: The plants, *E. deciduum*, *E. argentinum*, *E. myrsinites*, *E. cuneifolium*, *E. amplifolium*, *E. cuspidifolium*, *E. vaccinifolium*, *E. barbatum*, *E. laeteirrens*, *E. mucronatum* and *E. revolutum*, were collected during summer and winter in the states of south and northeast of Brazil. Using methodology recommended by the United Nations (UNODOC) was obtained the extracts of alkaloids. The extracts were eluted in LC/MS and GC/MS and analyzed the fragmentations. **Results and discussion**: The evaluation preliminary of the fragmentations suggest presence the alkaloids: benzoylecgonine, ecgonine, hidroxytropanone, tropanone, methylecgonine, tropacocaine and others, these not are present in all species. *E. argentinum* and *E cuneifolium* have more of these alkaloids. Have differences in metabolism phytochemical with the seasons in these species. **Conclusions**: All species synthesize, in summer and winter, alkaloids of the ecgonine. The biosynthesis of these alkaloids is changed between the species and the seasons.

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AN 20- AMOXICILLIN AND CEPHALEXIN STABILITY IN URINE SAMPLE FOR THE PURPOSE OF REUSE IN AGRICULTURE

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Introduction: The human urine can be used as an agricultural fertilizer, cause it is rich in substances that are essential to the soil and plants. The human urine reuse in the agricultural requires attention by the drugs presence, like the antibiotics in function of the development of the microbian resistence beyond the known environmental impacts. Recently, in our laboratory a method for simultaneous determination of amoxicillin (AMX) and cephalexin (CFX) in urine samples was validated, one time that both of them are excreted unchanged in the urine in high rates than 80% of the administered dose. **Objectives:** asses the AMX and CFX stability under storage different conditions. **Materials and Methods:** was used the validated method by High Performance Liquid Chromatography, in the following conditions: column C18 (250 x 4,6 mm; 5um), precolumn C18 (21 x 4,6; 5um); flow of 1,0 mL.min-1; mobile phase composed by methanol; acetate buffer 1-mM (22:78 v\v) and injection volume of 25uL; detector wave-length 265 nmUV. The sample preparation consisted in a simple dilution 1:100 with Ultra pure water. Ampicillin was the internal standard. The samples were evaluated in different conditions: room temperature evaluated for 24 hours, in refrigerator stored for two weeks and three freeze-thaw cycles. In all tests was evaluated three concentrations of both antibiotics, so the CFX concentrations were 5, 10 and 25 µg.mL-1 and 110, 140 and 170 µg.mL-1 to AMX. All the analyzis was performed in triplicate. As acceptance criteria, was used a 10% low variation, relative to a zero time determination. **Results and discussion:** In room temperature CFX only 25 µg.mL-1 has shown results lower than limit. To the AMX, all the concentrations have a 10% low variation, relative to the zero time determination. For refrigerator storage, the stability was similar for CFX and AMX. On the freeze-thaw tests to the CFX, at the lowest concentration, all the cycles has been above of the limit. At the 10 µg.mL-1, the first and second cycles has been above the limit, the third was into the acceptance criteria and in the highest concentration, all the cycles was into the established limit. To the AMX, in the freeze-thaw cycle, all the cycles has a 10% lowest variation. **Conclusion:** The CFX has shown stability in the different conditions evaluated only for the highest concentration; the AMX has shown stable for all concentrations tested and in three different conditions. A possible explanation is that the AMX concentrations are higher about the CFX. Thus, it is believed that when in higher concentrations, the analyte shows higher stability or in lower concentration there is a higher probability of analytical error and the variation exceeds the established as acceptance criteria.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 21– DEVELOPMENT AND VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRIC METHOD FOR THE DETERMINATION OF URACIL AND DIHYDROURACIL IN DRIED BLOOD SPOTS

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Introduction: Fluoropyrimidine toxicity has been related to a deficiency on its metabolic clearance mediated by the dihydropyrimidine dehydrogenase (DPD) enzyme. The identification of patients with reduced DPD activity is mostly based on the measurement of the concentrations of the endogenous compound uracil (U) and its DPD metabolic product, 5,6-dihydrouracil (UH2) in plasma samples, either endogenous or after a loading oral dose of U. Due to the intrinsic stability and handling safety of dried blood spots (DBS), as well as its facilitated sampling and transportation, the determination of U and UH2 in DBS after an oral loading dose of U is a promising alternative to evaluate DPD activity in patients scheduled to receive fluoropyrimidine-based chemotherapy. **Objective:** The aim of this study was to develop and validate a method for the determination of U and UH2 in DBS, after and oral loading dose of U, using LC-MS/MS, to be used in the evaluation of DPD activity. **Materials and Methods:** Two 8 mm DBS were cut and added with 1 mL of methanol containing the IS (5-FU 1.0 ng mL^{-1}) to a polypropylene micro tube, incubated 15 min at 40 ºC and 1000 rpm. The organic extract was evaporated to dryness and recovered in 75 µL of water, 25 µL was injected on a LC-MS/MS system. Separation was performed in a C18 column (150 x 2.1 mm, 1.7 µm) at 10 °C. Mobile phase was a mixture of acetic acid 0.5% (v/v) and acetonitrile (gradient 96:4 to 50:50, v/v) at a flow rate of 0.2 ml min^{-1}. Monitored transitions for quantitation were m/z: 113/70 for U, 115/55 for UH2 and 131/114 for IS. Method was applied to 7 paired DBS and plasma samples obtained 2 hours after a 1 g oral dose of uracil. Estimated plasma concentrations were obtained from DBS after adjustment by red blood cell/plasma partition and individual Hct using the formula $C_{plasma}=C_{DBS} / [(1-Hct) + (K_{RBC/plasma}*Hct)]$. **Results and Discussion:** Retention times were 2.7 for U and UH2 and 3.0 for IS. Calibration curve presented a quadratic response from 0.2 to 20.0 µg mL^{-1} with r=0.99. Mean extraction yield from DBS was 73% for U and 74% for UH2, accuracy 97 to 104%, intra-assay precision 5.97 to 13.79% and inter-assay precision 6.18 to 9.87%. No significant ion suppression effect was observed (≤11%). The Hct effect on IM measurement tested in Hct ranging from 25 to 50%, presented acceptable results (89 to 113% of nominal values). U and UH2 DBS concentrations in clinical samples ranged from 0.38 to 7.91 µg mL^{-1} and 0.87 to 5.82 µg mL^{-1}. Estimated plasma concentrations and metabolic ratios were comparable to those obtained from measured plasma: 104 ± 15% for U, 105 ± 14 % for UH2 and 100 ± 12% [UH2]/[U]. **Conclusions:** A LC-MS/MS method for the determination of U and UH2 in DBS was developed and validated. The procedure has adequate analytical performance and can be an efficient tool to identify patients with reduced DPD activity.

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AN 22- PROTENTIAL ACCUMULATION OF PROTOPANAXADIOL-TYPE GINSENOSIDES IN SIX-MONTHS TOXICOKINETIC STUDY OF SHENMAI INJECTION IN DOGS

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Introduction: SHENMAI injection (SMI) is an herbal injection widely used in China. Ginsenosides are the major components of SMI. **Objective:** To monitor the exposure level of SMI during long-term treatment, a 6-month toxicokinetic experiment was performed in dogs. **Materials and methods:** Twenty-four beagle dogs were dived into four groups: a control group (0.9% NaCl solution), a low-dosage group (2 g/kg), a medium-dosage group (6 g/kg), and a high-dosage group (20 g/kg). Groups were i.v. infused with vehicle and SMI daily for 180 d (6 mo). Blood samples for analysis were collected at specific time points as follows: pre-dose (0 h); at 10, 30, and 60 min during infusion; and at 10, 30, 60, 90, 120, 240, and 300 min post-administration. Concentrations of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 in the plasma were determined simultaneously by liquid chromatography–tandem mass spectrometry. **Results and Discussion:** Non-compartmental parameters were further calculated and analyzed. Significant differences were found between the kinetic behavior of 20(*S*)-protopanaxadiol-type (PPD-type) and 20(*S*)-protopanaxatriol-type (PPT-type) ginsenosides. **Conclusion:** Increasing in the exposure level of PPD-type ginsenosides was observed in dogs during the experiment. Therefore, PPD-type ginsenosides are closely related to the immunity modulation effect of SMI. PPD-type ginsenoside accumulation must be carefully monitored in future SMI research.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 23- SIMULTANEOUS DETERMINATION OF HIPPURIC ACID, METHYL HIPPURIC ACID, MANDELIC ACID , PHENILGLIOXILIC ACID AND CREATININE IN URINE BY ULTRA PERFORMANCE ION PAIRING LIQUID CHROMATOGRAPHY

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INTRODUCTION: Organic solvents are widely employed in chemical and pharmaceutical industries. As they are generally volatiles, the air on working environment can be contaminated, allowing their inhalation by workers. Exposure to high concentrations of volatile organic compounds causes adverse health effects such as acute and chronic respiratory disorders, functional disorders of the central nervous system, skin irritation and chromosomal aberrations.
The control of exposure of some solvents occurs by monitoring their metabolites in urine. It is widely accepted that hippuric acid, methyl hippuric acid, mandelic acid and phenilglioxilic acid are biological indicators of toluene, xylene, ethylbenzene and styrene respectively. In this context the measurement of creatinine is important because in a random sample, urinary analytes can be normalized by the creatinine concentration to account for the variation in urinary concentrations between subjects.Usually, creatinine values are measured using spectrophotometric methods, and then the amount of the others analytes is determined by chromatographic methods. Creatinine is a very polar small molecule, which is not easily retained by reverse phase column in chromatography. The addition of an ion pairing agent, in others words, an oppositely charged counter-ion will form a complex less polar than the original compound changing significantly the creatinine retention time. **OBJECTIVE**: The aim of the present study was to develop and validate a simple, fast, reliable and cost-effective methodology for the simultaneous determination of creatinine and four biological indicators of exposure by Ultra Performance Ion Pairing Liquid Chromatography. **METHODOLOGY:** Previously, the urine sample was diluted (1:20) with ultrapure water. A amount equivalent to 3mM of 1-octanesulfonic acid was added in the mobile phase (phosphate buffer 25mM pH=2.1 /methanol 90:10) as ion pairing agent. It was used an UPLC® Acquity BEH C18 column and the run time was 5.0 minutes. Absorbance was measured at 225 nm and 254 nm. **RESULTS:** The method was linear (r>0.99) for the five compounds in the range of 0.5 to 3.0 g/L. The measurement of the five analytes showed accuracy (90%-110%) e precision (RSD < 10%). The quantification limit was inferior then 0.025 g/L to all substances. **CONCLUSION:** Thus, the proposed method proved to be adequate for the simultaneous determination of urinary hippuric acid, methyl hippuric acid, mandelic acid, phenilglioxilic acid and creatinine, resulting in a great economy of time.

AN 24- SIMULTANEOUS DETERMINATION OF ETHANOL AND ACETONE IN URINE AND PLASMA BY HEADSPACE GAS CHROMATOGRAPHY AUTOSAMPLER AND FLAME IONIZATION DETECTOR - GC/FID-HS.

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Introduction: The routine monitoring of industrial workers to check exposure to potentially toxic components, and the detection of endogenous metabolites in the body in certain clinical diagnoses, are extremely important. The simultaneous determination allows more efficiency and speed in the release of the report for the patient, also reducing analysis costs. **Objective:** The aim of this study was to validate a simple, rapid, sensitive and low cost method for the simultaneous determination of acetone and ethanol in urine and plasma by headspace gas chromatography with flame ionization detection. **Methods:** The method consists in a simple extraction of both analytes off the sample by evaporation, and sampling the vapor above the fluid (plasma, urine or others) after reached thermal equilibrium gas in a closed vial of 22 mL. The volatilized components present in headspace are aspirated by a syringe and injected into chromatograph. To improve the extraction and reach more accurate results it was developed a diluent solution using 2-butanol as an internal standard and a salt of ammonium sulfate to turn the solution saturated, a technique called salting-out. A simple dissolution of an inorganic salt in water can decrease the solubility of an organic substance in water and consequently increase its volatility. The extraction involves the addition of 5 mL diluent solution, containing the internal standard, and 100 µL of the sample, standard, or control into a vial. The vial is shaken for 30 seconds on vortex. Chromatographic separation was performed on PerkinElmer BAC 1: 450°C: 30m x 320µm x 1.8 µm column and mobile phase Nitrogen 99,999%. The chromatographic running time was approximately 4 minutes. **Results:** The parameters evaluated in the validation were selectivity, linearity, accuracy, precision, repeatability and reproducibility, detection limit, quantification limit and matrix effect. For ethanol the calibration curve was linear with r^2>0.9995. Analytical linear range was from 1.0 to 210.0 mg/dL. The limits of detection and quantification were 0.2 and 0.6 mg/dL, respectively. The accuracy (94.2-102.2%), intra-assay precision (1.6-3.6%) and inter-assay precision (3.1-3.5%) were acceptable. For acetone, the calibration curve was linear with r^2>0.9995. Analytical linear range was from 5.0 to 300.0 mg/L. The limits of detection and quantification were 0.3 and 0.9 mg/L, respectively. The accuracy (89.0-106.4%), intra-assay precision (0.9-4.9%) and inter-assay precision (7.3-9.9%) were acceptable. For both analytes the matrix effect was not observed. **Conclusion:** In conclusion, the GC/FID-HS method has been developed successfully for monitoring industrial workers and the simultaneous quantitative analysis of acetone and ethanol.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 25- THE DEVELOPMENT AND APPLICATION OF ANALYTICAL METHODS FOR THE DETECTION OF STIMULANTS IN ADULTERATED NUTRITIONAL SUPPLEMENTS

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Introduction: The fraudulent addition of active pharmaceutical compounds in nutritional supplements is, indeed, a world problematic. Often, it can be found several advertisements on various supplement packaging assuring weight loss, increased intellectual and/or physical capacity and sexual stimulation. These may have been 'spiked' with synthetic drugs containing formulations which are apparently harmless to users. In Brazil, data availability about adulteration of nutritional supplements is scarce. Therefore, it becomes important to study the presence of stimulants in these products. **Objective**: The development and application of analytical methods using chromatographic techniques for the detection, identification and quantification of stimulants undeclared on the labels of dietary supplements. **Material and Methods**: Samples of food supplements were obtained from specialized supplement stores and through websites from all over the state of São Paulo. The evaluated extraction techniques were: accelerated solvent extraction (ASE), followed by solid phase extraction (SPE), QuEChERS technique (modified method) and the extraction technique with methanol. The analysis of stimulants (caffeine, fenproporex, amfepramone, fenfluramine, sibutramine, phentermine, ephedrine, phenylpropanolamine, pseudoephedrine, diphenylamine, 4- metilhexan -2-amine, synephrine, metilsinefrina, desmethylsibutramine and didesmethylsibutramine) in nutritional supplements was gas chromatography coupled with a nitrogen–phosphorus detector (GC-NPD). **Results and Discussion**: From all evaluated extraction techniques, it was chosen the extraction with methanol as it allowed for the detection of the desired active ingredients as well as the fact that it required a very small quantity of sample, solvent and stimulant standards. GC-NPD has been tested for the detection of caffeine, fenproporex, amfepramone, fenfluramine, sibutramine, phentermine, ephedrine, phenylpropanolamine, pseudoephedrine diphenylamine and it yielded satisfactory results as it was capable of separating the active principles without overlapping. From the five supplements analyzed, three of them gave a positive result for caffeine and sibutramine according to the chosen methodology. **Conclusions**: The ASE technique followed by SPE did not permit a full analysis of the substances subjected to extraction, hence, this method was not suitable for the evaluation of supplements. The QuEChERS technique did not provide an adequate response due to the high interference from the solvent used as it resulted in the full suppression of two substances of interest. Consequently, extraction with methanol has proven to be the most suitable one.

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AN 26- ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF ZINC-PROTOPORPHYRIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: PARTIAL RESULTS.

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Introduction: Exposure to lead (Pb) compromises the heme synthesis in a few steps, one of them is iron incorporation into protoporphyrin IX structure by ferrochelatase. Pb inhibits this enzyme causing zinc to be preferably incorporated to protoporphyrin IX, forming zinc protoporphyrin (ZnPP), a natural metabolite of this process and considered a biological marker of effect for the evaluation lead exposure. **Objective**: The aim of this study is to validate the determination method of blood ZnPP by high-performance liquid chromatography equipped with fluorescence detector. **Materials and Methods**: Total blood collected in EDTA tubes was used. Two hundred microliters of blood were extracted with 1,0 mL of ethyl acetate-acetic acid (80:20) and 30 µL of supernatant were injected into the sytem. Mobile phase was acetic acid 30% and methanol (88:12 v/v). The excitation and emission wavelengths were 425nm and 593nm, respectively. At this current stage, only the analytical methods' precision and accuracy were evaluated as figures of merits. **Results and Discussion**: The total time of the chromatographic run was 12 min, the ZnPP retention time of 9.4 min. Comparing the calibration curve prepared in a buffered solution and another one in blood, it was observed that in this last one the recovery of spiked samples was on average of 107% (±12.7) while in the one in buffered solution were 166% ((± 2,2) . The coefficients of variation for 10, 50 and 100 mg/dL spiked samples were 9,5; 1,5 and 4,8%, respectively. Analyzing the replicates of spiked samples one day after their preparation, there was a drop of about 50% of the detection signal of those prepared in blood, which was not observed in those prepared in buffer solution. In the chromatograms it was observed the presence of two peaks, one of them being the ZnPP and the other one that might be the free protoporphyrin, which will be investigated. **Conclusion**: A new method will be tested with possible insertion of neutral buffered solution. Literature data indicates that zinc in acid media dissociates from protoporphyrin, allowing only the detection of its free form. The next steps of the validation process will include the analysis of blood lead and ZnPP levels in exposed populations and in the control group.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 27- COMPARATIVE VALIDATION OF A PROCEDURE FOR THE DETERMINATION OF CARBAMAZEPINE IN SERUM BY PROTEIN PRECIPITATION

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Carbamazepine is an antiepileptic drug used in the treatment of different types of epileptic seizures. The treatment of epilepsy is chronic and prophylactic, in which is indispensable the serum monitoring of this drug, to achieve the desired drug concentration to avoid seizures and the adverse effects of this medication. To obtain a sensitive, accurate, rapid execution and low cost method, was comparatively validated a methodology for determination of carbamazepine in serum, using a protein precipitation technique followed by a simple liquid-liquid extraction. The sample preparation consists in add 300 µl acetonitrile in 300 µl of serum, followed by homogenization for 5 seconds and centrifugation in 2500 rpm, the supernatant was injected in the chromatograph. The chromatographic running time was approximately 8 minutes. Chromatographic separation was performed on Pump Waters Model 515, Waters autoinjector Model 717, and Waters UV-Vis detector model 489. From the results obtained, the methodology of protein precipitation was considered comparable to the methodology already used in the Toxicology Department, which uses the technique of liquid-liquid extraction. The parameters evaluated were relative standard deviation (RSD = 4.91%) and recovery (Recovery = 96.6%). Furthermore, there is a direct comparative analysis in which the method was confronted with each other on an equivalent curve, named Passing & Bablok method. This method has been based on the ideal case, where the methods correlate a curve with R = 1, where X = Y, which the slope of the curve equal to 1 and the intercept with the abscissa is zero. The regression curve obtained from this method was: ConcNew Method= 1,0554ConcOld Method- 0.5812 and r = 0.991. It was observed from the curve analysis that the slope was close to 1.0 and the linear coefficient was close of 0, indicating that a method is comparable to the other. Another analysis used was the Bland-Altman's, in this technique was plotted graphs of distribution of absolute and percentage differences between the two methodologies. From these dispersion graphs was concluded that the difference between the results from the two methods are not significant for the treatment since, mostly, they are shown near their mean (m = 0.17 ug / mL). In addition, almost all results were concordant with respect to the interpretation of clinical outcome of the patient, according to the therapeutic range of 4-12 µg /mL. With the data obtained and the statistical analysis it was concluded that the developed method presented a lower cost and a faster execution. Futhermore it also presents reliable and consistent results with the current methodology, and provides greater safety for the operator, since exposure occupational chlorinated solvents was avoided.

AN 28- DEVELOPMENT OF SCREENING METHOD FOR THE DETECTION OF PSYCHOACTIVE SUBSTANCES IN HAIR SAMPLES USING IMMUNOLOGICAL TECHNIQUES

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Introduction: Currently, hair is being recognized as the third main biological matrix for analysis of drugs of abuse, preceded by urine and blood. The main characteristic of hair is that it comprises of a wider detection window compared to other matrices which allows the estimation a chronological profile consumption according to the length of the hair to be analyzed. In Brazil, the number of assays using hair as a matrix of choice is increasing. In general, toxicological analysis of drugs of abuse are conducted by screening followed by confirmatory techniques, for positive results. Screening techniques include immunochemical assays which are not only faster but also cost-effective. **Aims:** In this study, a screening method is being developed for psychoactive substances in hair from the adjustment of immunoassay blood kit DOA I WB P with the Biochip technology - Randox Laboratories®. **Material and Methods:** Artificial hair will be developed by incorporation of the following model compounds: amphetamines, barbiturates, benzodiazepines, cocaine , opiates and THC. This will allow the evaluation of different methods of extraction and digestion of hair matrix reported in the existing literature. To evaluate the results, all samples will also be analyzed by GC-MS confirmation methods for all analytes. Analytical validation of the immunoassay will be performed according to parameters such as sensitivity, specificity, positive predictive value, negative predictive value, accuracy, limit of detection and precision. **Results and Discussion:** The described immunoassay method has been tested for amphetamines, through alkaline digestion followed by liquid-phase microextraction (LPME) and also for cocaine and morphine, through incubation of the matrix in methanol overnight. Both experiments have showed satisfactory results, nevertheless, new methods will be evaluated making use of the artificial hair such as incubation with acetonitrile, acidified organic solvents, buffer solutions, solvent mixtures and acidic extraction. Confirmatory methods, which will be used to verify the results of the immunoassay, have been developed and will be fully validated according to international guidelines. **Conclusions:** The adjustment of the immunoassay blood kit (DOA I WB P) to hair samples appeared to be promising for the detection of drugs of abuse. Not only because it comprises some of the basic requirements for screening methods as cross-reactivity with the parent drug and their metabolites and high sensitivity, but also due to the satisfactory results given by the previous experiments.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

AN 29- MERCURY RECOVERY STUDY IN URINE BY TECHNICAL CV-AAS AND HG-ICP-OES

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Introduction: Metallic mercury can be found in the workplace, where workers are exposed to chronic intoxication by inhalation and eventually, when there is accidental leaks. For this reason, the Ministry of Labor establishes the NR-7 a maximum allowable value (VMP), it is extremely important monitoring, in order to prevent diseases related to metal and ensure the health of workers during their working lives. One of the analytical techniques used to determine mercury in urine is the hydride generation coupled to optical emission spectrometry inductively coupled plasma (HG-ICP-OES) and the generation of cold vapor / hydride coupled with atomic absorption spectrometry (CV-AAS). These equipments show good detection and is recommended for this analysis. **Objective:** To evaluate the recovery of mercury in urine, using the techniques of HG-ICP-OES and CV-AAS through interlaboratory study programs. **Materials and Methods:** To evaluate the recovery were analyzed three samples of interlaboratory program, in duplicate, being digested in the digester microwave, Provecto brand Analytics. Subsequently, read in CV-AAS, Agilent, model FS and HG 280 AA-ICP-OES, Agilent, model ES-720 with analytical curve in the range 1.0 to 10 µg / L. We used the Z-score index and accuracy of calculation for approving the results. **Results and Discussion:** The results of the mercury recovery remained in the range of 90-110% and the values found for the Z-score were with index <2 for both equipments. These results were considered satisfactory according to the DOQ-CGCRE-008 Revision 04 - JUL/2011 Inmetro. **Conclusion:** The methodologies applied in the quantification proved to be effective because they have acceptable recovery, with the possibility of meeting the VMP established in the regulatory regulation of the Ministry of Labor (NR-7). It is worth mentioning also that the technique HG-ICP-OES proved to be more advantageous than the technique of CV-AAS due to less time reading and less interference in the analysis which makes it more attractive for use in routine laboratory.

References:

1- EPA 7470A - Mercury in Liquid Waste (Manual Cold-Vapor Technique)
2- NR-7 – Programa de Controle Médico de Saúde Ocupacional.
3- DOC-CGCRE-008 – Orientação Sobre Validação de Métodos Analíticos.

AN 30- COMPARATIVE STUDY OF SAMPLE PREPARATION METHODS FOR THE DETERMINATION OF PESTICIDE RESIDUES IN BABY FOOD EMPLOYING GC-MS

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Introduction: Several analytical methods have been developed for multiresidue analysis of pesticide in baby foods, which have been designed to meet the maximum residue limit (MRL) of 0.01 mg kg^{-1} fixed by European Community. Most of these methods includes sample preparation based on QuEChERS (quick, easy, cheap, effective, robust and safe), which involves a liquid-liquid partitioning and subsequent cleanup by dispersive solid-phase extraction (d-SPE), however, the low enrichment factor of extract represents the main disadvantage of this method, when compared to other sample preparation methods such as the dispersive liquid-liquid microextraction (DLLME). **Objective:** In order to satisfy baby food safety requirements, the d-SPE and DLLME were evaluated and compared with reference to achieve low LODs and LOQs, minimize matrix effects, and consequently obtain quantitatively accurate results for the determination of pesticide residues in baby food employing gas chromatography-mass spectrometry (GC-MS). **Materials and Methods:** Twenty-four pesticides of 12 chemical classes were studied. The acetate-buffered QueChERS procedure was employed for pre-concentration of the analytes. From QuEChERS extract, the procedures d-SPE and DLLME were carried out and then compared. For d-SPE procedure, 1mL of extract, 50mg of C_{18}, 50mg of PSA and 150mg of $MgSO_4$ were vortexed, centrifuged, and then analyzed by GC-MS. For DLLME, 1mL of extract (disperser solvent) was mixed with 75µL of CCl_4 (extraction solvent) and the mixture was rapidly injected into 4mL of deionized water, then shaken, centrifuged, and the bottom layer was analyzed. **Results and Discussion:** The DLLME achieved analytical response between 2 and 9 times higher than d-SPE. The d-SPE provided better cleanup of extract, consequently lower matrix effect was obtained with this procedure. Using DLLME, the LOD for most of the pesticides was 0.005 mg kg^{-1}, and the LOQs varied between 0.01 and 0.05 mg kg^{-1}. Employing d-SPE, the LODs and LOQs were between 2 and 10 times higher than those obtained with DLLME. A good linearity ($r > 0.99$) was verified from 0.01 to 2.0 mg kg^{-1} and between 0.1 and 2.0 mg kg^{-1} for DLLME and d-SPE matrix-matched calibration curves. Recoveries between 72% (diuron) and 124% (phosalone), and from 55% (dichlorvos) to 143% (azinphos-methyl) were obtained with DLLME and d-SPE procedures, respectively, and RSD values ≤ 20%, under repeatability and within-reproducibility conditions. **Conclusions:** Compared to d-SPE procedure, the DLLME achieved satisfactory performance criteria for the studied pesticides as well as provided analytical selectivity and sensibility enough to meet the baby food safety requirements, including LODs ≤ 0.01 mg kg^{-1}; however, higher matrix effect was observed for all compounds.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

TOXINOLOGY

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

TO 01— THE INFLUENCE OF MEDIA REPORTS ON CALLS RECEIVED AT THE TYGERBERG POISON INFORMATION CENTRE REGARDING SPIDER BITES

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Introduction: There are currently more than 40 000 species listed on the World Spider Catalogue, but only a few are of medical importance. Various factors beyond medicine and arachnology play a role in the medical effects of spiders on humans; journalism is only one of them. **Objective:** This study aimed to determine the influence of media reports on calls received at the Tygerberg Poison Information Centre (TPIC) regarding spider bites. **Materials and Methods:** A retrospective analysis of the TPIC database was conducted over a four year period (January 2010 – December 2013). Media reports were obtained from a web search as well as the archives of all major South African newspapers and the media group 'Media24'. Calls pertaining to spider bites were compared 30 days prior to and 30 days after publication of any article covering spider bites. **Results and Discussion:** The TPIC received 25 510 calls during the study period of which 2.6% (n=661) related to spider bites. Most of these calls (72.5%, n=479) were received from the general public. Spiders were witnessed in a third of suspected spider bite cases of which only 10% (n=65) could be identified. Most patients presented with local swelling (25.7%, n=170), pain (18.3%, n=121), and redness (17.1%, n=113). Antivenom was advised in 34 cases (5.1%). An increase in calls after publications in nationally distributed newspapers or magazines were seen (Median difference = 9; p=0.03). Spider bite articles in individual provincial publications did not result in a statistically significant change in call-volume (median calls prior to an article = 14; median calls following an article = 16; p=0.216). History pertaining spider bite is often unreliable and a spider in the vicinity does not equal a spider bite. Necrotic arachnidism is over-diagnosed and is often a convenient diagnosis for unexplained local tissue or dermal problems. Most of the articles were sensationalised and not verified, leading to misinformation greatly fuelling the mythology surrounding spider bites. **Conclusion:** Nationwide media reports on spider bites did raise the number of calls to the TPIC. Poison centres should be prepared for such a possible influx in calls. The diagnosis of spider bites should only be made with substantial evidence to help debunk the myth surrounding spider bites.

TO 02- STRUCTURAL CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF BORDONEIN-L, AN L-AMINO ACID OXIDASE ISOLATED FROM *Crotalus durissus terrificus* SNAKE VENOM

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Introduction: L-amino acid oxidases (LAAOs) are flavoenzymes that catalyze the oxidation of L-amino acids, concomitantly producing hydrogen peroxide and ammonia. They are usually present in low amounts in snake venoms (sv) and their roles are still poorly understood. However, sv-LAAOs are relevant in key biological activities (e.g., antitumor, antiviral, antiparasitic, antibacterial and inhibition/activation of platelet aggregation), being potential leads for therapeutics. **Objectives**: The aim of this study was to perform the exhaustive structural characterization of bordonein-L, an L-amino acid oxidase from *Crotalus durissus terrificus* venom (CdtV), and evaluate its cytotoxic and anti-leishmanicidal activities. **Materials and Methods:** Bordonein-L was isolated from CdtV through 3 chromatographic steps (ionic exchange, molecular exclusion and affinity chromatography) and its purity was checked by reversed-phase chromatography (RPC). The LAAO activity was measured according to Kishimoto and Takahashi method (*Anal. Biochem*. 298:136, 2001). The cellular viability of fibroblasts was determined after cell stimulation with different concentrations of bordonein-L. The anti-leishmanicidal activity was evaluated against *Leishmania amazonensis* after 24 h of incubation with LAAO. To characterize the sequence of LAAO, the enzyme was subjected to an Orbitrap Elite™ Hybrid Ion Trap-Orbitrap Mass Spectrometer, using HCD and ETD fragmentations, after digestion with different proteases, including a newly isolated N-terminal arginine lysine protease. The data were searched against a database downloaded from Uniprot ('L-amino acid oxidase' and '*Crotalus*' used as keywords) using the error tolerant search engine ByonicÔ and manual *de novo* sequencing. **Results and Discussion:** Bordonein-L was successfully isolated from CdtV and showed cytotoxicity upon fibroblasts over 0.23 mUAE which might suggest a role during snake envenoming. Additionally, bordonein-L induced the death of *L. amazonensis* promastigotes. RPC and MS analysis showed that at least 2 LAAO isoforms are found in CdtV. The amino acid sequence of bordonein-L was deduced through the 'bottom-up' and 'middle-down' strategies and it showed high sequence similarity compared to other LAAOs sequences. Bordonein-L also showed diagnostic ions for glycopeptides (HexNAc and Hex-HexNAc) upon HCD fragmentation and evidences for one N-glycosylation and some O-glycosylation sites. **Conclusion:** Bordonein-L is a glycoprotein and the major LAAO isoform present in CdtV. The enzyme shares a high level of sequence identity with other LAAOs deposited in data banks. It displayed toxicity upon fibroblasts and *L. amazonensis* promastigotes and its cytotoxic activity might be one of its roles during the envenoming by snake bite. *Support:* FAPESP

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

TO 03- INHIBITORY EFFECTS OF PHENOLIC COMPOUNDS ON THE FIBRINOGENOLYTIC ACTIVITY INDUCED BY SNAKE VENOMS

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Introduction: Proteases present in snake venoms, which produce their noxious effects by degradation of proteins like fibrinogen, fibrin, collagens, and factors from coagulation cascade, presents great importance in cellular morphology, transportation, remodeling and repairing tissues, and clot formation. Therefore, the use of natural substances capable of inhibit at least partially the local envenomation effects has been growing. Among these substances, the phenolic compounds class, broadly distributed in nature, presents many attested beneficial effects such as immunomodulation, anticarcinogenic, antiophidian properties and cell membrane protection. **Objective:** In this context, the phenolic compounds vanillic acid (Va), epicatechin (Ep), p-cumaric (pC) acid and catechin (Ca) were used in an attempt to inhibit completely or partially the proteolytic effect of venoms, from *Crotalus durissus terrificus (C.d.t.), Bothrops atrox (B. atrox) and Bothrops jararacussu (B. jussu.*) snake species, over fibrinogen. **Material and Methods:** Each snake venom (10µg/µL mv^{-1}) and phenol were incubated in two doses, 1:1 and 1:0.5 (w/w, crude venom: phenolic compound), for 30 minutes at 37°C, after that the 80µg of fibrinogen were added and let incubating for 90 minutes at the same temperature. Afterwards, the samples were denatured and submitted to polyacrylamide gel electrophorese with sodium sulfate dodecyl (SDS-PAGE) to the visualization of results. **Results and discussion:** For *C.d.t.* venom, Va (1:1) inhibited mostly of the proteolysis, whereas Ep (1:1) and Ca (1:1) presented a lesser inhibition and pC did not inhibited. For *B. atrox* Va and Ep showed moderated inhibitory effects at 1:1 dose, while pC and Ca did not inhibited proteolysis. For *B. jussu.* venom, Va and Ca showed a moderated inhibition, while Ep and pC did not inhibited proteolysis. **Conclusion:** These results showed the inhibitory effect of phenolic compounds on proteolysis induced by venoms. The nature of the inhibition cannot be confirmed, however, it may be suggested the direct ligation of the phenols on the proteases or a protector effect on the fibrinogen molecules. For both hypothesis there are works supporting them. More studies must be done with these compounds in order to understand these effects, and hereafter recommend them as auxiliary in the conventional serum therapy.

Financial support: FAPEMIG and CNPq

TO 04- REGISTRATION AND LOCATION OF BITIS GABONICA IN ANGOLA

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The available literature reports the existence of Bitis Gabonica in Angola, but restricts its location to some northern points, however specimens were found in different parts of Angolan geography, which are reported in this work. As part of the study of poisons, poisonous snakes and snakebites in Angola, took place the "Ndala Lutangila" Expedition that traveled five provinces of Angola, the activity aims: The capture of live snakes to study your poison and promotional work and awareness aimed at health professionals and the general public, preventing the snake bites, the most neglected of neglected tropical diseases, according to WHO. During the expedition were found five copies of Bitis Gabonica, which are added to the two existing at the Research and Information for Drug and Toxicology Center (CIMETOX) showing a wide distribution in Angola.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

TO 05- EPIDEMIOLOGY OF SNAKEBITES IN ANGOLA

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An observational, descriptive and retrospective research was done from January 2013 to October 2014, about the snakebite cases recorded by the Research and Information of Drugs and Toxicology Center of Malanje, Angola, to assess their snakebite behavior and some of its main features. It was considered the sociodemographic variables sex and age of the patients as well as the accident place and the clinical features. The number of reports in this period was 51 cases. It was observed that snakebites affected mainly child whith age between 0 and 10 years (31.8%), followed by the individuals whose ages were between 11 and 40 years old, representing 27.3% of the cases. Referring to gender, men were the most affected (54.5%) but there was no statistically significant differences as compared with women. Regarding the demographic distribution of cases reported, the provinces with the highest proportion of cases reported were Malanje with 27.2%, Uige with 22.8% and Luanda with 18.2%. Other provinces reported a negligible number of cases. Swelling and pain were the most common signs and symptoms followed by local bleeding, blistering and bruising. Systemic bleeding, hypotension, compartment syndrome and death were infrequent complications. Neurotoxicity showed up a few times. The parts of the body most affected were the lower and upper limbs. The accidents mostly occurred while performing job or roam the countryside.

TO 06- UNIDENTIFIED BOTHROPS ENVENOMATION IN AN TOXICOLOGY ASSISTANCE CENTER IN BRAZIL. THE LACK OF HEALTH INFORMATION LEADING TO FEWER ACCURATE DIAGNOSIS.

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Introduction: Among the poisonous animals accidents, snake bites are the most importante due to it's high level of complexity and morbidity. The World Health Organization considered snake bites as a neglected tropical disease on account of the low identification and notification of cases as well for the lack of health information and policies in the management of these situation. In Brazil snake bites are the second most prevalent accident and the leading cause of deaths associated with poisonous animals intoxication. The Bothrops (BTP) genus its responsible for 80% of all symptomatic snake bite events in Brazil, being the leading cause of permanent sequel and the second most lethal snake envenomation. Identification of the animal and its clinical presentation can change the treatment and the prognosis of the patient. **Objective:** To identify possible BTP envenomations on patients with a symptomatic snake bite event, between identified and unknown animals, that sought assistance in a toxicology assistence service. **Materials and Methods:** All snake bite acidents cases attended in the period between october-2013 and june-2015, were studied and stratified in the animal especies and presenting symptons. From the symptoms presented, the botropical possible manifestations (pain, edema, hyperemia, local bleeding, equimoses, hematuria, hematemeses and petechiae) were choosen to select the possible BTP acidents among symptomatic cases. The patients were divided in two groups being Possible Botropical Envenomation (PBE), patients that presented one or more BTP symptoms, and Non-Tipical Botropical Presentation (NTP), patients with other symptoms or asymptomatic. **Results and Discussion:** A total of 89 cases were obtained, being 71 symptomatics and 18 asymptomatics. From the total of cases 22,4% (n=20) were inicially defined as a BTPs acident, 19,1% (n=17) as rattlesnake, 1,1%(n=1) as Surucucu, and 57,3% (n=51) as an unkown or unidentified snake. 26 diferent symptons were related in all events, among the 8 chosen BTPs symptoms 46%(n=41) of the patients presented pain, 40,4%(n=36) edema, 14,6%(n=13) equimoses, 12,3%(n=11) hyperemia, 3,3(n=3) hematuria, 2,2%(n=2) local bleeding, and 1,1%(n=1) presented hematemesis and petechiae. 29,4% (n=5) of the rattlesnake cases and 54,9%(n=28) unkown cases were considered as PBE. After the analisis 58,4%(n=52) were classified as PBE (73,2% of the symptomatic events) and 41,5% (n=37) as NTP with a greater assemblence with the typical epidemiology. **Conclusions:** The lack of health information on snake envenomations leads to lower rates of acurate diagnosis of the BTP envenomations, since the number of BTPs accidents may be higher than the inicialy reported (a diference of 36%) considering the symptoms presented.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

TO 07- PURIFICATION FROM CRUDE VENOM AND CONSTRUCTION OF A RECOMBINANT VECTOR OF TS8, A NEUROTOXIN OBTAINED FROM *TITYUS SERRULATUS* SCORPION VENOM.

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Introduction: The *Tityus serrulatus*, known as yellow scorpion, is the most dangerous specie found in Brazil. Its venom is composed by several different components, particularly neurotoxins which act on ion channels and are responsible for neurotoxic effects on envenoming. These neurotoxins have been widely used in the discovery and study of ion channels, as well as in treatment of diseases associated to them. Unfortunately, only small amounts of toxins can be obtained from scorpion venom. Therefore, one alternative to obtain them in larger quantities is to perform their cloning and expression in heterologous systems. **Objectives:** The aims of this study are the purification and the cloning of Ts8, one neurotoxin which acts on K^+ channels. **Material and Methods:** The purification of Ts8 in the native form was performed in three chromatographic steps. Firstly, 50 mg of *Tityus serrulatus* venom was submitted to an ion exchange chromatography. Secondly, the fraction VIIIb obtained from the first step was submitted to a reversed-phase chromatography on a C18 column. Finally, the fraction 13 from second step was rechromatographed on a C-18 reversed-phase column for peptides. The fractions obtained in this last chromatographic step were analyzed by MALDI-TOF mass spectrometry. Furthermore, the cDNA encoding the Ts8 sequence was obtained through analysis of library from *Tityus serrulatus* venom gland. Specific primers were designed containing cleavage sites for XhoI/Kex2 and NotI restriction enzymes. The PCR products and the expression vector pPICZαA (Invitrogen) were digested with the restriction enzyme, applied on 1% agarose gel and purified. The ligation of the vector with the gene was performed by reaction with the enzyme T4 DNA ligase for 16h at 16°C. The recombinant plasmid was used for transformation of *Escherichia coli* DH5α cells by heat shock, plated on LB medium containing 25 mg/mL Zeocin and incubated at 37°C, for 16h. **Results and Discussion:** Three chromatographic steps used were able to purify theTs8 and the isolation of Ts8 was confirmed by mass spectrometry analysis. The mass of the detected ion (m/z) of 6715 Da corresponds to Ts8. Moreover, the recombinant clones obtained on a transformation with *E.coli* cells were subject to DNA sequencing to confirm the insert presence. Subsequently, it will be subject to transformation in *Pichia pastoris* for heterologous expression. **Conclusion:** This study presents the isolation of Ts8 and a strategy for cloning this toxin, which will be important to produce the recombinant neurotoxin expressed in heterologous system. Further functional analysis between the native and recombinant forms will be performed aiming future applications.

Acknowledgments: CAPES, CNPq, FAPESP, NAPTOXAN for financial support.

TO 08- NEUROPROTECTIVE EFFECTS OF LOW MOLECULAR WEIGHT FRACTION OF *BOTHROPOIDES JARARACA* SNAKE VENOM AGAINST H_2O_2-INDUCED APOPTOSIS IN HIPPOCAMPAL CELLS

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Introduction: The bradykinin potentiating peptides (BPPs) from the low molecular weight fraction (LMWF) of *Bothropoides jararaca* (*Bj*) venom were the first natural inhibitors of angiotensin-converting enzyme described. It has been demonstrated that BPPs are capable of positively modulating the catalytic activity of argininosuccinate synthase (AsS), increasing the production of L-arginine and consequent production of polyamines, which are essential to growth, differentiation, cell proliferation, and neuroprotection. **Objective:** As the low molecular weight fraction (LMWF) of the *Bj* crude venom (CV) contains multiple active biologically active peptides, including BPPs, the aim of the present study was identify the neuroprotective property of LMWF in cultured hippocampal cells. **Materials and methods:** To confirm the absence of proteins, the LMWF filtrate was analysed by electrophoresis in SDS-PAGE (16%), mass spectrometry and FT-IR. To perform primary hippocampal culture, Wistar neonate brain hippocampus was dissected, processed and cells were plated. To test cytotoxicity, the cell cultures were treated or not with 0.001, 0.01, 0.1, 1 and 10 µg/mL of LMWF for 06, 12, 24 and 48 h. Cell stress was promoted using H_2O_2 50 µM for 20 hours. Neuroprotection was evaluated by concomitant treatment with LMWF (0.1µg/mL) and H_2O_2 (50 µM). Cell viability was determined using MTT assay. Expression of cleaved caspase -3 was performed by western blot. In the moment, we are doing RT-PCR analyses to evaluate the expression of SOD1, caspase-8 and 9. Quantitative values corresponding to relative viability were analysed by one-way ANOVA for statistically significant differences between groups (Turkey's post-test, respectively; $p<0.05$). **Results and discussion:** LMWF filtrate contained no high-molecular weight (HMW; >10kDa) contaminants compared to CV. Mass spectrometry and FT-IR analysis confirmed the absence of HMW components in the filtrate. LMWF no show toxicity in all conditions tested After the treatment with 50 µM of H_2O_2 we showed a decrease of cell viability of 32,84±8,06% ($p<0,001$), while the previous treatment in culture cells with 0,1 µg/mL LMWF following by 50 µM of H_2O_2, it was observed 98,26 ± 6,8% ($p<0,05$) of cell viability. Evidence has shown that cells treated with LMWF 0.1 µg/mL and then with H_2O_2 (50µM) show lower expression of cleaved caspase -3 cells than the cells treated just with H_2O_2. Microscopy images showed a number of cells in the field and maintenance of cell morphology, characterized by the presence of long axons in the cells previously treated with LMWF, compared to cells treated only with H_2O_2. **Conclusion:** In summary the results suggest that LMWF has cytoprotective activity against oxidative stress caused by H_2O_2 (50µM).

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

TO 09- ACUTE DELIBERATE ORGANOPHOSPHATE (COUMAPHOS) POISONING WITH INTERMEDIATE SYNDROME IN A ONE YEAR OLD CHILD

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Objective: To report a case of acute organophosphate poisoning in a one year old child and development of intermediate syndrome in just eight hours after exposure. Case Report: A case of a previously well one year old child who came in the emergency room because of sudden onset of difficulty of breathing, cyanosis, excessive oral secretions, one episode of diarrhea and weakness noted three hours earlier after ingesting allegedly contaminated powdered milk formula. Vital signs showed a blood pressure of 90/60, cardiac rate of 112 per minute, respiratory rate of 12 cycles per minute and axillary temperature of 35.4 degrees Celsius. ABG analysis showed respiratory acidosis. Endotracheal intubation was immediately done and was hooked on mechanical ventilation. The toxidrome of the patient is compatible with acute cholinergic excess; hence a trial dose of atropine was given with some improvements. Eight hours later, neurological examination showed absence of deep tendon reflexes, no spontaneous respiration, no response to pain, flaccid muscle tone, no neck rigidity and lateralizing signs but with spontaneous eye opening. RBC cholinesterase determination showed a result of 0.057 delta pH/hr, which is significantly depressed. Atropine was given at 0.02 mg/kg intravenously until full atropinization was achieved. Packed RBC transfusion was given.Twenty-four hours later, patient was noted to have response to painful stimuli and spontaneous respiration. A repeat RBC cholinesterase determination showed a result of 0.25 delta pH/hr. Atropine was continued as needed. Test was done on the allegedly contaminated milk using GC-MS, it is positive for Coumaphos. The patient was discharge after seven days. Conclusion: Intermediate syndrome usually develops within 48-96 hours after acute cholinergic crisis due to prolonged inhibition of cholinesterases.

TO 10— STUDIES OF A FRACTION OF THE SNAKE VENOM *Crotalus durissus collilineatus* CROTAMINE-NEGATIVE: EVALUATION OF THE ANALGESIC, ANTI-INFLAMMATORY AND CYTOTOXIC ACTIVITIES.

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Introduction: The crude venom of *Crotalus durissus collilineatus* snake promotes neurological signs and symptoms in their accidents, and victims report analgesia at the site of the sting, without tissue destruction. It comprises crotoxina, gyroxin, convulxin and crotamine. The crotamine is a basic myotoxin which is expressed in different populations of non-uniformly rattlesnakes. The crude venom is unsuitable for therapeutic use, so the fractionation is incentive. In a recent study, the crude venom was fractionated on preparative HPLC-PDA and the results indicated that a fraction (Fr5) of *C. d. collilineatus*, the crotamine-negative type, may have analgesic activity by suppressing the nociception induced by acetic acid. **Objectives:** Study the mechanism of action of the Fr5 fraction, of the snake *venom Crotalus durissus collilineatus* crotamine-negative. **Methodology:** Was evaluated the antinociceptive effect by acetic acid test (0.6%), in mice; acute toxicity, by up and down method; Identifying the molecular weight of the fraction, Fr5 in the spectrometer MALDI-TOF operating in the positive linear mode using external calibration and phospholipase test. **Results and discussion:** The Fr5 (40 µg / i.p.) was able to reduce by 47% the number of contortions (i.p.) and 87% (p.o.), compared to control. The assessment of acute toxicity showed no morbidity and no mortality (dose of 1000 µg / kg, po). The study showed a fraction component of 25,679 Da and showed no phospholipase activity. **Conclusions:** The Fr5 fraction, may present analgesic activity by suppressing the nociception induced by acetic acid. Suggesting be related to effects on spinal peripheral sites. Displays reduced acute toxicity values in mice. It does not present phospholipase activity and presents component of 25679 Da.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

TO 11- CASE REPORT: DIFFERENCE IN THE EVOLUTION OF SCORPION ENVENOMATION INVOLVING SIBLINGS OF DIFFERENT AGES

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Introduction: The state of Minas Gerais has the biggest number of scorpionism notifications in Brazil, almost all cases caused by Tityus serrulatus. About 90% of accidents are characterized as mild, 9% as moderate and 1% as severe, and children under 6 years old and / or low weight children have higher risk of severity. **Objective:** Clinical case description. **Case report:** LLSS, 6 years old, 20kg and RJSS, 4 years, 15 kg, brothers, were admitted to hospital 90 minutes after being stung by the same scorpion. The oldest, stung twice and before the younger brother had local pain, no other symptoms, treated only with analgesia. Was kept in observation for 12 hours, without any complications. Was discharged from the hospital the next day. The other had presented four episodes of pre-admission vomiting, presented to hospital admission sweating, with bradycardia, tachypnea, but no other changes to the breath sounds. We administered 4 ampoules of anti-scorpion serum. Evolved with worsening of respiratory pattern, crackles in the right lung, and was transferred to the ICU intubated. Presented to the ICU with mixed shock (cardiogenic? distributive?). Presented repolarization changes in the electrocardiogram and the echocardiogram showed diffuse hypokinesia of the left ventricle with preserved systolic function. Evolved with progressive improvement with intensive care, and was discharged after nine days of hospitalization. **Discussion:** The clinical picture varies greatly depending on the scorpion species, quantity and age of the patient. Evaluations should be continuous because the clinicalpresentation is dynamic and the severity classification can change even after specific serum administration. Very severe cases usually need intensive and specialized care. Even though the older brother was stung multiple times and before the youngest, he evolved more favorably, which calls attention to the importance of age in the prediction of prognosis and need for more aggressive treatment.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

SOCIAL TOXICOLOGY

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

SC 01- INTEGRATED MONITORING NETWORK OF LABORATORIES OF AGROCHEMICALS (REMILA) - A STRATEGY TO EXPAND THE ASSESSMENT OF EXPOSURE TO AGROCHEMICALS IN BRAZIL

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Introduction: Brazil is the world's largest consumer of agrochemicals. The heavy use of these products results in the contamination of soil, air, water and food that, in turn, influence the safety and food sovereignty, human health and ecosystems. The agrochemicals interfere in physiological mechanisms of support of life common to human beings, associated with a wide range of damage to health, e.g. neurotoxic effects and cancer. In this way, the surveillance plays a key role in the prevention and mitigation of the risks associated with its high use and contamination. Thus, the laboratory structure of the country plays a strategic role as it represents the technical-scientific and legal support required to take the effective actions. **Objective:** To propose the creation of a national Integrated Monitoring Network of Laboratories of Agrochemicals (REMILA) for the detection and quantification in water, food and environmental and clinical samples. **Materials and Methods:** Research conducted in freely accessible national databases that bring together laboratories with accredited methods for the analysis of agrochemicals between September 2013 and October 2014. The information obtained allowed perform, in November 2014, a workshop to present the nationally analytical laboratory capacity to the representatives of the laboratories identified and Health Ministry, enabling to debate about the main existing demands and possible strategies of programs for the monitoring of contamination by agrochemicals. An enhanced discussion via a digital platform is currently taking place, providing guidelines for the deployment of the REMILA. **Results and Discussion:** The research showed the existence of 58 laboratories (public and private); 78% located in the Southeast region, 15% in the Southern, 5% in the Midwest and 2% in the Northeast. 739 active ingredients were identified and 105 veterinary products distributed in 182 chemical groups, analyzed into the main groups of matrices, represented by food, environmental and clinical. It was noted the possibility of expansion of existing programs that analyze products of plant origin and water, as well as the need to implement new programs for the monitoring of processed products and of animal origin, as well as the clinical and environmental samples. It was noticed the importance in investing in accreditation of laboratories in the North and Northeast regions and set surveillance networks according to regional specificities based in the information about agricultural, livestock activity, consumption, toxicity and notification of intoxication.**Conclusion:** The implementation of the REMILA is necessary and feasible, and may contribute to the evaluation of impacts of agrochemicals and mitigate the damage to human and environmental health.

SC 02- THE CRACK COCAINE EFFECTS IN THE ORGANISM AND ITS PSYCHOSOCIAL IMPACT

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Introduction: Data of II National Survey about Alcohol and Drugs in 2012 set forth that 1,8 million Brazilian adults used crack cocaine (CRC) in some moment in his life. Brazil has the world's largest market of CRC. The Brazilian Health Ministry reported that in 2011 there were 2 million crack users (CU), of which about one-third obtained the cure of addiction, a third followed consuming and a third died, 85% of deaths from violence. **Objective:** To analyze the action of CRC in the organism and its psychosocial repercussion. **Methods:** A literature review through research in electronic databases Micromedex e Pubmed was perfomed about CRC; were also consulted guidelines published by government agencies for the management of CU. **Results and Discussion:** The CRC has cardiotoxic effects, accounting for the high prevalence of acute myocardial infarction in young patients. Arrhythmias are also frequent and result from the blocking action of sodium gate. The "lung crack" is the most important lung disorder and is characterized by fever, dyspnea, hypoxemia, alveolar infiltrates on x-ray and eosinophilia. Neurological and psychiatric complications constitute the greatest impact, such as cognitive deficits and substance dependence (SD), bringing important social repercussions. CRC use during the pregnancy can cause preterm labor, low birth weight and neurobehavioral changes. The treatment of acute intoxications and withdrawal syndrome consists in support and symptomatic measures; special attention to hyperthermia, electrolytic and cardiac disorders. The treatment of SD is complex and should be multidisciplinary, addressing physical, psychological, social and legal issues. The psychosocial interventions should involve cognitive behavioral therapy, social abilities training and relapse prevention. Treatment should also include family approach and neuropsychological rehabilitation. **Conclusion:** Previous psychiatric disorders (PD) are associated with an increased risk of drug use. Furthermore drug abuse also contributes to developing PD. These situations are more than health problems, these are major social issues. The mental health care in Brazil is still far from ideal, leading to social exclusion of PD patients. In recent years the incidence of female CU increased, most on reproductive age. The addicted pregnant women do not accomplish properly prenatal, in addition to exposing the fetus to harmful substances and infections. The unwanted pregnancy may still result in one more negative consequence, the child abandonment. The sum of marginalization of patients with PD and CU's child exacerbates the social environment, contributing to increasing urban violence, constituting another Brazilian public problem.

A | R | T
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SC 03- AYAHUASCA (SANTO DAIME TEA) CAN BE USED AS AN AUXILIARY SUBSTANCE FOR THE TREATMENT OF ABUSE DRUGS USERS?

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Introduction: Current literature describes several reports of clinical, psychological, and epidemiological evaluations made on adepts of the religious practices that utilizes the hallucinogen beverage more known as Ayahuasca (Aya) or Santo Daime tea, which has been used by licit and principally illicit abuse drug users. Amphetamine is known by their high potential of provoking addiction, anxiety and locomotor activity increase. **Objective:** evaluate if Aya modifies drug-addiction and behavioral effects caused by the amphetamine, an abuse substance globally used, principally amongst adolescents. **Material and Methods:** Experiment 1- adolescent rats were trained to ingest water and amphetamine solution (0.6 mg/ml), in the absence or presence of Aya (2ml/kg, gavage). The ingestion index of Amph solution represents rat addiction. Experiment 2- adolescent rats received: amphetamine (Amph, 5 mg/kg, i.p., 5 days), Aya (2ml/kg, gavage), or Amph plus Aya. The locomotion and anxiety behaviors were evaluated using Open Field (OF) and Elevated Plus Maze (EPM) apparatus. In the OF, the fear, emotionality, and the anxiety produced by a novel ambient make the animals avoid passing through the center of the apparatus and increase their grooming behavior. These parameters are utilized as an anxiety index to evaluate the drug exposition effect. Additionally, the sensitization due amphetamine exposition cause an increase in the locomotor activity of the rat. In the EPM, the number of entries in closed arms represents an index of anxiety. **Results and Discussion:** In the first experiment was observed that the rats preferred Amph solution over water, and that Aya decreased significantly (22.5%) the addiction for it. In the EPM, Aya decreased significantly the percentage of closed arms entries caused by Amph. In the OF, Aya normalized the hyperlocomotor effect and the grooming behavior in the animals that received Amph, as well as the latency time and the number of crossings in the center of the arena. Reports show that religious use of Aya exerts various beneficial effects in humans, however scientific evidence using experimental animals are still rare in the current literature. In this study, Aya was able to mitigate Amph effects. Therefore, it presents strong scientific evidence for beneficial effects of Aya in individuals using abuse drugs. **Conclusion:** These findings are an indicative that Ayahuasca may be considered a potential auxiliary instrument to enhance the effects of clinical therapies in cases of drug abuse.

The present work was supported by CEATOX.

SC 04- PREVENTION PROGRAM DRUGS OF ABUSE AND VIOLENCE "GUARDIAN ANGELS" 2014.

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Introduction: Currently are reported numerous events related to the frequent use of drugs and the increasingly early youth violence, as well as sexually transmitted diseases (STDs). Considering the many risk factors for the use and abuse of drugs highlight the lack of information on the effects and harms of drugs in the body. **Objectives:** to verify through use of questionnaire the knowledge of students about the harmful effects of abuse drugs before and after an awareness lecture. **Method:** Firstly graduate students in biology, biomedicine and nursing were training to be monitors able to inform (teachers, students of primary, secondary and higher education, and other institutions) through lectures about the harmful effects drugs, prevention, reducing violence and STDs. Secondly a pre questionnaire and other post lectures (20 questions each (multi-choice tests) to evaluate the effectiveness of learning were applied. The external public of the lectures were: students of elementary school (n=1580) and medium (n=550) Public Schools, institutions of young offenders (n=120) - CASA Foundation, units of Botucatu SP and Iaras - SP; companies requesting talks on tobacco and alcohol and other drugs, for example - Internal Week for Prevention of Industrial Accidents as examples: SENAC (n=120), SESI (n=40) among other segments [eg Churches (n=100), War of Tyre (70) PROERD - Program (600) and JCC Military Police (300), Narcotics and Alcoholics Anonymous (17), Home Adolescent Manain (12)]. **Results and Discussion**: Students acquired knowledge appropriately as found by the highest percentage of correct answers of 35 ± 15% pre to 88 ± 12% post-lectures. Post-lectures demonstrating effectiveness in training as well as the proposed development of theme lectures issue with timing of explanation. **Conclusion:** It was observed in the evaluation of questionnaires pre and post-talks the significant improvement of the information about drugs and STDs, as well as teachers' comments mentioning the importance of the program with monitors properly trained as an essential tool in the prevention of drugs, reducing school violence and the incidence of sexually transmitted diseases.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

SC 05- CHARACTERIZATION OF PSYCHOTROPICS RELATED PROBLEM INTENSITY OF SCHOOL AGE TEENAGERS

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Introduction: the use of psychotropic substances is a complex phenomenon which involves multiple factors and poses a serious problem for public care. It leads to severe emotional and physical consequences, which endangers proper maturing of teenagers and compromises their future role in society. **Objective:** to identify the intensity of problems caused by the use of psychotropic substances by teenagers at high school and basic education levels from schools located in popular areas, according to a socio-occupational typology determined for a city in southern Brazil. **Materials and methods:** this is a descriptive epidemiologic study of transversal design. The study proposal was presented to the local Regional Nucleus of Education and later to school principals. A document attesting that all subjects had been clarified about the study and that they were participating of free-will was given to all involved students and later collected after their responsible guardians signed it. DUSI was the tool of choice for data collection. The study was submitted to the Brazilian National Ethics Committee for research involving humans and was approved in accordance with Resolution n° 466/12 under number CAAE 18400813.8.0000.0104. **Results and discussion:** three schools on the chosen area participated in the study, and 79 forms were gathered. Lie Scale analysis excluded 23 forms, being considered valid for the study only 20 forms from the original total. By analysing problem intensity it was verified that students from both basic and high education show higher indexes of behaviour patterns. The teenagers tend to display conduct deviations, diagnosed by evidence of disrupting behaviours and involvement with dangerous and/or illicit activities. Antisocial behaviours tend to persist, evidencing a lacking ability to learn from negative experiences and recognize own faults. Conduct deviations are frequently associated with low learning rates at school and relationship problems with peers. Risk behaviours involving sexual activities, drug abuse and even suicide attempts as means to "alleviate frustrations" are frequent. **Conclusion:** the behaviour patterns of psychotropic using teenagers were considered to be the problem of greater intensity. Also, the lack of recreational access, professionalizing courses and local sports areas leaves the teenagers unoccupied when not in school, predisposing drug abuse. This picture stimulates the discussion regarding the existing fragilities of society and contributes to the debate of public politics, mostly at educational areas.

Acknowledgements: Decit/SCTIE/MS; CNPq; Araucaria Foundation and SESA-PR for their support.

SC 06- EXPOSURE PROFILE OF TEENAGERS IN BASIC AND HIGH EDUCATION TO PSYCHOTROPIC SUBSTANCES

MUNHOZ G.R.[1], REIS L.C.[1], STEINBACK C.[1], GARCIA L.C.[1], MIRANDA B.C.[1], RUBIM M.[1], MENOTTI V.S.[1], BIONDARO L.D.[1], MOSSINI S.A.G.[1], NISHIYAMA P.[1]

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Introduction: nowadays, the use of psychotropic substances is explored by teenagers as means to facilitate socialization. Depression, guilt, low self-esteem and other forms of psychic suffering and emotion-related issues add to the problem. Teenagers not only tend to seek new experiences, but are also easily influenced by their peers and many feel the need to "escape" personal difficulties or simply defy the moral values stipulated by their parents. All of these fragilities lead to easier drug abuse. **Objective:** to describe the exposure profile of teenagers coursing both basic and high education at schools on a popular area, according to a socio-occupational typology determined for a city in southern Brazil. **Materials and methods:** this is a descriptive epidemiologic study of transversal design. The study proposal was presented to the local Regional Nucleus of Education and later to school principals. A document attesting that all subjects had been clarified about the study and that they were participating of free-will was given to all involved students and later collected after their responsible guardians signed it. DUSI was the tool of choice for data collection. The study was submitted to the Brazilian National Ethics Committee for research involving humans and was approved in accordance with Resolution n° 466/12 under number CAAE 18400813.8.0000.0104. **Results and discussion:** three schools on the chosen area participated in the study, and 79 forms were gathered. Lie Scale analysis excluded 23 forms, being considered valid for the study only 20 forms from the original total. The majority of the evaluated students belong to basic education and are females (62.5%) of age ranging from 10 to 13 years old (87.5%). Data show a considerable use of alcohol among the students (36.4% at basic education and 63.6% at high education). The use of anaesthetics was also reported at both levels of education. The use of the following drugs was also reported (a single case each): inhalants at basic education and marijuana and cocaine at high education. High alcohol consumption rates can be explained due to the fact that alcohol is a licit drug labeled as "social", such as tobacco, which facilitates peer acceptance of physically and psychologically troubled teenagers. **Conclusion:** this study evidences the consumption of drugs, mostly alcohol, by teenagers at early ages. This picture stimulates the discussion regarding the existing fragilities of society and contributes to the debate of public politics, mostly at educational areas.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

SC 07- EXPOSURE PROFILE OF TEENAGERS IN BASIC AND HIGH EDUCATION TO PSYCHOTROPIC SUBSTANCES

MUNHOZ G.R.[1], REIS L.C.[1], STEINBACK C.[1], GARCIA L.C.[1], MIRANDA B.C.[1], RUBIM M.[1], MENOTTI V.S.[1], BIONDARO L.D.[1], MOSSINI S.A.G.[1], NISHIYAMA P.[1]

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Introduction: nowadays, the use of psychotropic substances is explored by teenagers as means to facilitate socialization. Depression, guilt, low self-esteem and other forms of psychic suffering and emotion-related issues add to the problem. Teenagers not only tend to seek new experiences, but are also easily influenced by their peers and many feel the need to "escape" personal difficulties or simply defy the moral values stipulated by their parents. All of these fragilities lead to easier drug abuse. **Objective:** to describe the exposure profile of teenagers coursing both basic and high education at schools on a popular area, according to a socio-occupational typology determined for a city in southern Brazil. **Materials and methods:** this is a descriptive epidemiologic study of transversal design. The study proposal was presented to the local Regional Nucleus of Education and later to school principals. A document attesting that all subjects had been clarified about the study and that they were participating of free-will was given to all involved students and later collected after their responsible guardians signed it. DUSI was the tool of choice for data collection. The study was submitted to the Brazilian National Ethics Committee for research involving humans and was approved in accordance with Resolution n° 466/12 under number CAAE 18400813.8.0000.0104. **Results and discussion:** three schools on the chosen area participated in the study, and 79 forms were gathered. Lie Scale analysis excluded 23 forms, being considered valid for the study only 20 forms from the original total. The majority of the evaluated students belong to basic education and are females (62.5%) of age ranging from 10 to 13 years old (87.5%). Data show a considerable use of alcohol among the students (36.4% at basic education and 63.6% at high education). The use of anaesthetics was also reported at both levels of education. The use of the following drugs was also reported (a single case each): inhalants at basic education and marijuana and cocaine at high education. High alcohol consumption rates can be explained due to the fact that alcohol is a licit drug labeled as "social", such as tobacco, which facilitates peer acceptance of physically and psychologically troubled teenagers. **Conclusion:** this study evidences the consumption of drugs, mostly alcohol, by teenagers at early ages. This picture stimulates the discussion regarding the existing fragilities of society and contributes to the debate of public politics, mostly at educational areas.

Acknowledgements: Decit/SCTIE/MS; CNPq; Araucaria Foundation and SESA-PR for their support.

SC 08- DRUG USERS CHARACTERISTICS AND ASSOCIATION WITH DEATH

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Introduction: The use of drugs is considered a serious worldwide social and public health problem and is a risk factor for trauma and injuries resulting from violence, for not transmissible chronic diseases, and temporary and permanent functional losses. **Objective:** To characterize the profile of drug users reported to a center of information and toxicological assistance in Northwest Paraná and correlate the occurrence of deaths. **Methods:** Descriptive and transversal study, with cases reported in 2010-2011. The data were compiled from epidemiological forms of toxicology case, filed in the center, and submitted to univariate analysis with chi square test. **Results and discussion:** It was found 339 cases, most male (87.3%), with basic education level (61.0%) and age group 60 or older (37.2%). The most commonly used drug was alcohol (83.8%), and most had chronic alcohol intoxication (89.9%). Statistical analysis showed a statistically significant association between the use of alcohol and the occurrence of deaths. There was a predominance of falls (12.4%) and physical aggression (12.4%), but higher mortality was related to cases of stab wound and firearms *($p < 0.05$)* with proportion of one death for each hospitalization. Liver cirrhosis (33.3%) and bleeding esophageal varicose veins (30.1%), accounted for more than half of the cases of death, with a significant association between death and the medical diagnosis of liver cirrhosis *($p = 0.01$)*. Most individuals (63.1%) remained hospitalized for a period of ten days, and the most frequent hospital sector was the attention to the emergency unit (67.0%), although it was found a high number of hospitalizations in the intensive care unit (15.6%), where most deaths occurred (53.5%). There was a significant association between the occurrence of deaths and short stay admissions at the attention to the emergency unit, and this association was protecting *($p = 0.0$)*. The deaths were associated with prolonged hospitalization (≥21 days) and admission to the intensive care unit. **Conclusion:** Despite being a documentary study, the presented data reinforce that the consumption of drugs of abuse influences morbidity and mortality, particularly in men age group 60 years old or more and low education. Mortality was significantly correlated with the use of alcohol, with stab wound and firearms, and liver cirrhosis, prolonged hospitalization, and hospitalization in intensive care unit.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

SC 09- INTENTIONAL CHEMICAL BURN AND VIOLENCE TO CHILDREN

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Introduction: Violence against children is a major public health problem in Brazil, and health services have an important role in prevention, diagnosis, notification and monitoring these cases. The information and toxicological assistance centers - CIAT act as sentinel, since they can identify cases of poisoning involving violence situations to children. **Objective:** To describe a case of intentional chemical burn on an infant who lives in a socially vulnerable family. **Materials and Methods:** Descriptive and documentary study, in a case study modality, with collected data from Toxicological epidemiological Occurrence record, filed at a CIAT in Northwest Paraná, which carried out the notification and followed the case. **Results and Discussion:** Infant, seven months, admitted to an Emergency Unit presenting burns on 65% of the body surface, and transferred to a pediatric intensive care unit of a hospital in the same city and, due to the severity of the case, forwarded the next day for a Burned Treatment Center - CTQ, where he was hospitalized for 68 days, receiving hospital discharge somewhat better. The burn was caused intentionally by the stepfather who, after discussion with the child's mother (a dysfunctional drug user for 20 years, 11 pregnancies and eight births), "poured" Thinner® at the place where the infant was asleep, and set fire. The monitoring of the case by the CIAT contributed to a quality initial care and the early transfer to a burned treatment center made it possible to continue the treatment with the necessary support to the patient´s care demands. **Conclusion:** The satisfactory outcome of the case shows the importance of referral services as CIAT and the CTQ to care complex cases. However, from the uniqueness of the case, it is possible to verify the inefficiency of public policies on accessing families in vulnerable situation.

SC 10- INTENTIONAL CHEMICAL BURN AND VIOLENCE TO CHILDREN

REIS L.M.[2], MESCHIAL W.C.[1,2], OKAMOTO A.R.C.[2], PIRES Í.H.V.[1,2], SILVA L.F.F.[2], GUERREIRO C.R.[1], SANTANA C.J.[1], OLIVEIRA M.L.F.[1,2]

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

SC 11- INTENTIONAL CHEMICAL BURN AND VIOLENCE TO CHILDREN

REIS L.M.[2], MESCHIAL W.C.[1,2], OKAMOTO A.R.C.[2], PIRES I.H.V.[1,2], SILVA L.F.F.[2], GUERREIRO C.R.[1], SANTANA C.J.[1], OLIVEIRA M.L.F.[1,2]

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Introduction: Violence against children is a major public health problem in Brazil, and health services have an important role in prevention, diagnosis, notification and monitoring these cases. The information and toxicological assistance centers - CIAT act as sentinel, since they can identify cases of poisoning involving violence situations to children. **Objective:** To describe a case of intentional chemical burn on an infant who lives in a socially vulnerable family. **Materials and Methods:** Descriptive and documentary study, in a case study modality, with collected data from Toxicological epidemiological Occurrence record, filed at a CIAT in Northwest Paraná, which carried out the notification and followed the case. **Results and Discussion:** Infant, seven months, admitted to an Emergency Unit presenting burns on 65% of the body surface, and transferred to a pediatric intensive care unit of a hospital in the same city and, due to the severity of the case, forwarded the next day for a Burned Treatment Center - CTQ, where he was hospitalized for 68 days, receiving hospital discharge somewhat better. The burn was caused intentionally by the stepfather who, after discussion with the child's mother (a dysfunctional drug user for 20 years, 11 pregnancies and eight births), "poured" Thinner® at the place where the infant was asleep, and set fire. The monitoring of the case by the CIAT contributed to a quality initial care and the early transfer to a burned treatment center made it possible to continue the treatment with the necessary support to the patient´s care demands. **Conclusion:** The satisfactory outcome of the case shows the importance of referral services as CIAT and the CTQ to care complex cases. However, from the uniqueness of the case, it is possible to verify the inefficiency of public policies on accessing families in vulnerable situation.

SC 12- SOCIAL AND HEALTH CONDITIONS OF ADEPT AGRICULTURISTS IN THE PLAN FOR RECONVERSION AND DIVERSIFICATION OF THE TOBACCO CULTURE IN THE STATE OF PARANÁ

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Introduction: The Framework-Convention for Tobacco Control was ratified in 2005 in Brazil, which disposes of articles 17 and 18 on matters related to "health and environment". Being the State of Paraná the third largest tobacco grower in the country, according to 2010/11's crop, amounting to a total of 148.140 tons with 36.110 families responsible for this production, in a total area of 69.630 ha, the Parana Department of Public Health came to terms with the Parana Department of Agriculture, to constitute a work group to elaborate a strategic plan for the reconversion and diversification of the agricultural production in tobacco growing proprieties, establishing a group of directives, amongst which one was specifically made to evaluate health and social conditions of these agriculturists. **Objective:** Identify the main risks to tobacco growers due to their exposure to nicotine which descend from the green tobacco leaf and pesticides. **Materials and Methods:** 15 families were selected in each of the 10 priority counties, where were applied individual questionnaires and then digitalized into Ministry of Health 's - DATASUS named FORMSUS. **Result and Discussion:** Participated in the diagnosis, 144 families (96%), amounting to 486 people interviewed. As for the education level, the highest percentage 49% (238) had the elementary school unfinished. 292 people (60%) admit being in direct contact with pesticides. Out of the total of people that admitted being in direct contact with pesticides, 50% show signs and symptoms related to the peripheral nervous system. 69 people (27,5%) recall to have shown some sign or symptom related to the cardiovascular system. The signs and symptoms related to the skin and mucous membranes added up to 44% (111 cases), followed by eye irritations with 23,5% and allergies 15,5%. The intoxication with nicotine, which can be found in the green tobacco leaf was referred by 29% (139 people), of which 56,8% have had headache , 28,1% muscular pain, 28,8% stomachache, 82% sickness, 79,9% weakness and 78,4% dizziness. **Conclusion:** The study demonstrates that the agriculturists' situation requires primary attention from "health surveillance" and "health education" and the development of actions related to management and social control, in order to minimize the risks of intoxication and disease related to the farming activity.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

SC 13- HEALTH EDUCATION: THE EXPERIENCE OF RIO GRANDE DO NORTE'S CENTER OF TOXICOLOGY ASSISTANCE

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Introduction: To teach health education goes beyond the curative assistance, it means to prioritize preventive measures and health promotion plus recognize that the beneficiaries of health services are individuals with their own thoughts and life standards and by that encourage them to fight for more life quality and dignity. The required interventions for health promotion must be oriented by group work and guaranteed by social policies that allow humanized and resolute assistance. Those interventions must allow the planning and development of educational activities in which families and communities are the aim of health care. To promote health it is necessary to bethink about it, considering it a changing concept that depends on social, cultural and historic values, allowing quality of life, fulfilling people's needs and aiming to establish health in its broadest sense.Thereby, is worth mentioning that Rio Grande do Norte's Center of Toxicology Assistance (CEATOX/RN) besides providing therapeutics conducts to the health professionals and beneficiaries, by the time of the accident that lead to an exogenous intoxication, also performs educational works. To spread the knowledge and to educate have a main role on people's everyday lives and enables a attention focused on the person, family and community, providing individual and collective changes in the biopsychosocial context of full health care with regard to prevention, damage reduction and habits changes to the welfare and population quality of life. **Objective:** Therefore this study aims to show the importance of CEATOX's health promotion actions at the Rio Grande do Norte Federal University's (UFRN) week of science, technology and culture (CIENTEC). **Materials and Methods:** For that, an exhibition stand was used for demonstrations about the main intoxicating products, household cleaning products, pesticides, coumarin, medicine, plant samples and demonstrative parts of venomous animals. In addition to it, informative flyers and brochures were handled to the public and institutions, warning about the risks of those products for the community. **Results and Discussion**: It was possible to see the success of the actions regarding the diffusion of the service of knowledge promotion in different institutions of society, plus the positive receptivity of the service by the scientific community and general public, leading to an increasing number of attendances by CEATOX in this period. **Conclusions:** Thus, it confirms the importance of health education works to people's participation on the improvement of life quality and collective health, considering the intersectoral partnerships for the improvement of assistance.

SC 14- ASSESSMENT OF GENETIC DAMAGE IN REPRODUCTIVE AGE WOMEN CAUSED BY ENVIRONMENTAL EXPOSURE TO PESTICIDES

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Introduction: Pesticide exposure and women's health information from developing countries is scarce and frequently not published in the international scientific literature. Forty-four percent of the total agricultural labor force in developing countries are female. Women may be occupationally- or environmentally exposed to pesticides; however, their perception of the risk is low. Many of the effects of pesticides in human health could be the same for males and females, but sex-related biological differences strongly support a distinct susceptibility to the toxic action of these chemicals. Besides, it should be considered that sometimes women are pregnant or accompanied by their children in an unhealthy environment. **Objective:** To evaluate the action of pesticides in women of reproductive age using biomarkers of exposure and genotoxicity. **Materials and Methods:** The variables used to describe pesticide exposure were butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), and to determine genotoxic effects were: comet assay in peripheral blood leukocytes (DI), their modification to determine oxidized pyrimidines using specific repair enzyme ENDO III (Endo sites) and the frequency of micronuclei in the buccal cells (MNBC). This study included 46 women from rural areas highly sprayed with pesticides and 101 controls from urban areas and without frequent use of pesticides in their homes. **Results and Discussion:** The average residence time was 28.00±8.00 years for women in rural areas and 29.77±2.55 years in urban areas ($p=0.871$). Both groups were similar regarding age, drinking and smoking habits, education level, and medicine consumption ($p\geq0.155$). The results showed a significant inhibition ($p<0.01$) of AChE in women from rural areas compared to controls but no significant modifications in BChE. We also found an increase in DNA damage ($p<0.01$) and oxidazed pyrimidines ($p<0.001$) but no differences were found in the frequency of MNBC ($p=0.85$). When compared with previous results of men exposed to pesticide mixtures women showed a significant decrease respect to workers ($p<0.001$) but significant increase respect to men environmentally exposed to pesticides ($p<0.01$), using comet assay. In respect to oxidative DNA damage we observed significant increase in relation to both occupationally and environmentally exposed men ($p<0.01$ and $p<0.001$, respectively). Gender-sensitive research is needed to properly address the study of women's pesticide exposures and related adverse outcomes. **Conclusions:** A better understanding of potential gender–environment interactions related to pesticide exposure and health effects in women is needed, highlight the importance of developing strategies to intervene and mitigate pesticides exposure, which could potentially reduce the incidence of health effects.

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A | R | T
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A | R | T
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OC 01- ANALYSIS OF GENOMIC DAMAGES AND GENETIC POLYMORPHISMS IN PUBLIC HEALTH AGENTS OCCUPATIONALLY EXPOSURE TO PESTICIDES AT GOIAS STATE, CENTRAL BRAZIL

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Introduction: Once, dengue fever is a public health problem, one of the most widely used mechanism to decrease the incidence of dengue cases is the use of pesticides. These products are used to eliminate mosquitoes and larvae and the endemic agents (CEAs) are occupationally exposed to pesticides. Changes in genetic material can be induced by substances contained in the pesticide formulations, which becomes important to evaluate the possible mutagenic, cytotoxic and genotoxic effects of pesticides for human health. **Objective:** To evaluate the influence of GSTT1 and GSTM1 polymorphisms and genomic damages caused by occupational exposure to pesticides, in endemic agents of Aparecida de Goiânia, Goiás, Brazil. **Methods:** 249 individuals, 161 (65%) occupationally exposed to pesticides and 88 (35%) nonexposed individuals were analyzed. For genomic damages, we used Comet test with the "Cometscore" version 1.5®. Samples of genomic DNA were obtained from the whole blood extraction with commercial kits according to the manufacturer's protocol. Analysis of GSTM1 and GSTT1 polymorphisms were detected using real time PCR. **Results and discussion:** The CEAs end up making one of most important workers occupationallu exposed to the effects of pesticides. The agents were exposed to 161 insecticides and larvicides, especially diflubenzuron (65%), novaluron (22%) malathion (12%) and deltamethrin (14%). The Olive tail (OTM), obtained by the comet assay demonstrated greater genomic damage in CEAs ($t = 6.468$, $p = 0.000$). Pesticides are potential mutagens and experimental data revealed that several pesticide ingredients have mutagenic properties. The analysis of GSTM1 and GSTT1 polymorphisms between control and case groups showed no statistical significance difference ($t = 1.248$, $p = 0.216$, $t = 0.732$, $p = 0.465$). In addition, there was no statistical difference when analyzing the GSTT1 and GSTM1 the polymorphism with OTM ($X2 = 0.196$; $p = 0.658$) between the exposed and control groups. We also analyzed the exposed group evaluating GST genotypes and the association between genomic damages and we detected a statistically association between GSTM1 null genotype and an increased genomic damage OTM ($p < 0.05$). **Conclusion:** Herein, the identification of effects caused by occupational exposure to pesticides by agents that deal against endemic diseases is of great importance, allowing for greater awareness of these workers to the harmful effects of exposure to pesticides.

OC 02- A GLOBAL INITIATIVE TO REFINE ACUTE INHALATION STUDIES THROUGH THE USE OF 'EVIDENT TOXICITY' AS AN ENDPOINT: TOWARDS ADOPTION OF THE FIXED CONCENTRATION PROCEDURE

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Submitted on behalf of the NC3Rs working group on the Fixed Concentration Procedure

Introduction: Acute inhalation studies are conducted in animals as part of chemical hazard identification and characterisation. Current accepted methods use death as an endpoint (OECD TG403 and TG436). The fixed concentration procedure (FCP) (draft OECD TG433) uses fewer animals and replaces lethality as an endpoint with 'evident toxicity.' Evident toxicity is defined as clear signs of toxicity that predict exposure to the next highest concentration will cause severe toxicity or death in most animals. The FCP was dropped from the OECD work plan in 2007 because of a lack of evidence for comparable performance with TG403 and TG436, suspected sex differences in toxicities (FCP originally only used females) and the ill-defined and subjective nature of evident toxicity. The first two issues have been resolved (Price et al., 2011; Stallard et al., 2011). **Objective:** A global initiative including 20 organisations, led by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has addressed the last concern with the aim of making evident toxicity more objective and transferable between laboratories. **Materials and methods:** Data on the clinical signs observed in individual animals in acute inhalation studies for 172 substances, carried out at two or more concentrations, were shared by the group. The data were analysed to determine whether there were any clinical signs (or combination of signs) observed in animals at a lower concentration that could predict toxicity at the next or higher concentration. For each sign (or combination of signs) positive predictive values (PPV) were determined. PPV is defined as the proportion of times that the presence of a clinical sign at the lower concentration is predictive of 'toxicity' (death of 2 or more animals) at the next highest concentration. **Results and Discussion:** The results suggest that signs such as body weight loss, irregular respiration, tremors and hypoactivity are highly predictive (PPV >90%) of severe toxicity or death at the next highest concentration. **Conclusions:** The working group has used these data to propose changes to TG433 that incorporate a clear indication of the clinical signs that define evident toxicity.

References:

Price et al., 2011. *Human & experimental toxicology*. 30:217-238
Stallard et al., 2011. *Human & experimental toxicology*. 30:239-249

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OC 03- DETERMINATION OF USAGE PROFILE OF AMPHETAMINES COMPOUNDS BY PROFESSIONAL DRIVERS OF A TRANSPORT COMPANY AT RIO GRANDE DO SUL STATE

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Introduction: The amphetamines – compounds belonging to the class of β-phenethylamine - are stimulant drugs of natural or synthetic origin, and those of synthetic origin are widely used by students, women and especially by drivers, which are utilized to increase the night-watching time. The indiscriminate use of these substances by professional drivers has spread in recent years, becoming a mean to cope with the strenuous working hours. However, the use of amphetamines has lead to reduced quality of life for drivers due to the toxic effects of the drug in the body . **Goals:** Establish the existence, profile and patterns of use of the stimulant by professional drivers of one transport company. **Materials and methods:** The study was cross-sectional analytical observational and it analized samples of 25 professional drivers from a private company with head-office located in Taquari Valley , a region in the state of Rio Grande do Sul, Brazil. The analysis of the biological samples (urine) was performed using the immunoassay method (RapidAmphetamine Test Bioeasy®). **Discussion and Results:** The company in which the survey was conducted has about 200 staffs as professional drivers, and with a verbal invitation, within them only 25 agreed to carry out the socio-epidemiological questionnaire and authorize the use of biological sample for analysis, thus reaching an average of 12.5%ofparticipatingemployess, all males aged between 26 and 55 years (38.2 ± 7.0). The questionnaire assessed possible factors that may be related to the use of amphetamine compounds, such as history of use, age, profession time, working hours, rest hours and level of education. Among the 25 professional drivers participating in this research(100%), which were submited to analyses to determine the amphetamine compounds, all showed negative results in their samples after applying the immunoassay. **Conclusions:** Based on the agreed data in the research and the analysis of biological samples, it can be concluded that even the drivers that showed negative results in the sample analysis, they mentioned in the survey that at a given moment have used some type of amphetamine compound. It valid to notes that the survey was conducted within a private company, which has important internal program which consists of driver training and fore most is governed by statutes and regulations.

OC 04- EXOGENOUS INTOXICATIONS IN OCCUPATIONAL ENVIRONMENT

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Introduction: At the same time with the modernization and the industrial advances in the world today, grow also the problems related to the health of workers, who each day are exposed to circumstances that affect their quality of life (1). Thus, the exposure to toxic substances in concentrations higher than those considered acceptable may lead to occupational poisonings. **Objective:** To study the occurrence and the profile of exogenous intoxication in the occupational environment in five regions of Brazil. **Methodology:** This is an epidemiological study, ecological analysis, carried out with data from the National System of Information Toxic Pharmacological (SINITOX) in the period from 2003 to 2012. Were included in this study, only the data for exogenous intoxications of occupational origin occurring in rural and urban zones of Brazil. **Results and Discussion:** In Brazil were found 62,427 cases of occupational accidents in the period studied. When evaluated the distribution of accidents by region, the majority occurred in the Southeast (43.5 %), followed by the South (31.9 %), Midwest (17.5 %), Northeast (6.3 %) and North (0.6 %). The average annual incidence of occupational poisonings in Brazil (3.3) and in the regions, the highest rates are observed in the Midwest (7.9) and South (7.3), followed by the Southeast (3.4), Northeast (0.7) and North (0.3). Among the agents that more caused the poisoning nationally, stand out the poisonous animals (40.1 %), pesticides (25.4 %), industrial chemicals (14.7 %) and household cleaning (6.4 %). In the Midwest, Northeast and South stood out accidents caused by poisonous animals, in the Southeast region have prevailed the accidents caused by pesticides and in the Northern region the predominance of cases was of chemicals for industrial use. Regarding the deaths were recorded 109 cases due to occupational poisoning in the same period, and the regions with the highest number were the Midwest (43.1%), South (25.7%), Southeast (15.6%), Northeast (11.9%) and again in smaller percentage the North (3.7%). **Conclusion:** The incidence of occupational poisonings over the Brazilian territory can change according factors such as the economic bases of each region, the importance given to the prevention of occupational risks and the reporting of these accidents. Due to the large number of intoxication observed in the work environment, it emphasizes the importance of studies, considering the local conditions to reduce its incidence through specific actions for each region.

References: (1) Levigard, Y. E. & Rozemberg, B. A interpretação dos profissionais de saúde acerca das queixas de 'nervos' no meio rural: uma aproximação ao problema das intoxicações por agrotóxicos. *Cad. Saude Publica* 20; 1515–1524, 2004.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OC 05- PROFILE OF OCCUPATIONAL POISONINGS REGISTERED IN COUNTY IN THE REGION OF MÉDIO ARAGUAIA, MATO GROSSO

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Introduction: The work environment often can bring damage to the health of the worker, when not respected the safety standards or preventive actions to minimize an inadequate exposure. **Objectives:** To study the occurrence and the profile of cases of occupational intoxication registered in the city of Barra do Garças, in the region of Médio Araguaia, Mato Grosso. **Methods:** We conducted a cross-sectional epidemiological study, with data obtained from the records of exogenous intoxication notified to the Notifiable Diseases Information System (SINAN) in Barra do Garças, from 2008 to 2014. **Results and Discussion:** There were 33 cases of occupational intoxication and we observed a prevalence of men (69.7%) over women (30.3%), prevalence of age groups between 30 and 39 years (27.3%) and 20-29 years (24.2%) and the most individuals were classified as the brown race (61.3%) or whites (32.6%). As for education, the majority (61.3 %) of the individuals do not have completed high school and 32.2% reached the middle school. Of the total number of accidents, 77.8% were intoxicated in urban areas and 22.2% in rural areas. The most frequently groups of toxic agents were pesticides (24.2%), chemical products for industrial use (18.2%), household cleaning (15.2%), food and beverages (15.2%), drugs (9%) and veterinary products (3%). In cases of poisoning caused by pesticides, the same occurred during the spraying (50%), dilution (12.5%) and seed treatment (12.5%). The route of exposure predominant among the cases was the respiratory (42.4%) and the digestive system (30.3%). Among the circumstances of the exposure, it is important to point out the accidental circumstances (30.3%), normal (27.3%), administration error (12.1%), attempted suicide (9.1%), environment (6.1%), and other (3%). Regarding the type of exposure, prevailed the only acute exposure (83.3%), followed by repeated acute (13.3%) and chronic (3.4%). **Conclusion:** The occurrence of occupational poisonings from pesticides by, in this city, has been a disturbing factor, especially in male individuals, who have proven to be the most affected in occupational accidents. The awareness of employees and employers, as well as the practices of prevention and safety are important tools to reduce the accident rates in occupational environment.

OC 06- OCCUPATIONAL EXPOSURE TO PESTICIDES, KNOWLEDGE AND PRACTICE OF POLICIES RELATED TO THE MANAGEMENT OF PESTICIDES, AND SEMEN QUALITY FROM URBAN SPRAYERS OF SOUTHEAST OF MEXICO.

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Introduction: Exposure to pesticides is associated with both acute and chronic health problems in farmers. A previous study by our group showed that farmers do not use personal protection equipment when handing pesticides and this exposure was associated with neurological symptoms, poor-quality semen, and damage to sperm DNA. In contrast, little is known about the exposure to pesticides of urban sprayers, in addition, policies and regulations related to the use and handling of pesticides is mostly for agricultural use and not for the control of urban pests. **Objective:** To gather information about occupational exposure to pesticides, about the knowledge and application of the policies related to pesticide use as well as to assess the activity of butyrylcholinesterase (BuChe) and semen quality in urban sprayers of Yucatan in Southeast of Mexico. **Material and Methods:** A cross-sectional, retrospective and comparative survey study was conducted. We studied 27 sprayers and 26 participants in the group without occupational exposure. A structured questionnaire was applied to participants. BuChE was used as a biomarker for organophosphorous (OF) and carbamates (CAR) pesticides and was obtained according to manufacturer instructions. Semen quality analysis was conducted according to WHO (2002). **Results and discussion:** The age in exposed group was 33.76 ± 9.34, vs 30.32 ± 5.25 years old in the unexposed group (p=0.11). Sprayers had worked in pest control for 4 years on average, they spraying pesticides for about 4 h/day, 6 days/week through all year round, joint mostly in the summer. A total of 18 active ingredients were used in the pesticides, the chemicals group identified were: pyrethroids, OF, coumarins, fenilpirazoles, CAR, chloronicotiniles and amides. Mask was used by 71% of the sprayers, while coveralls, waterproof gloves, boots and water resistant pants were worn by less than 50% of them. Ninety-six percent of sprayers think that pesticides use represents a health risk, and 76% consider spraying activity as dangerous. BuChE was similar among the groups, 9837.9±1844.5 U/L in sprayers *vs*. 10414.8±1558.4 U/L of unexposed group (p=0.24). This may be due to the fact that OF or CAR was used by only 26% of the workers. All semen quality parameters were similar among groups (p>0.05). Knowledge and application of policies were reported by only 30% of urban sprayers and were not associated with semen quality. **Conclusions:** This is the first study conducted in urban sprayers in our community. Although BuChE activity and semen quality were not different between groups, training programs aimed at the prevention of chronic effects of pesticides in urban sprayers are required.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

OC 07- DETERMINATION OF DIMETHYLFORMAMIDE METABOLITE IN RECENT URINE FOR EVALUATION OCCUPATIONAL BY SPE-RP HPLC -UV

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Introduction: Dimethylformamide (DMF) is a polar organic solvent, produced worldwide. It is widely used in the chemical industry in the production of various polymers, coating agents, inks, pharmaceuticals, adhesives, synthetic leather, among others. The DMF was identified by the Environmental Protection Agency and the Agency for Toxic Substances and Disease Register as a substance that is significant threat to human health. Occupational exposure occurs through inhalation of vapor and especially by direct contact with skin. After absorption, the DMF is primarily metabolized in the liver and its metabolites excreted in urine. The main metabolites of DMF are N-hydroxymethyl-N-methylformamide, N-methylformamide (NMF) N-acetyl-S- (N-metilcarbamil) cysteine. The presence of these metabolites is indicative of recent exposure to the solvent. **Objective:** This study aimed to validate determination of NMF in urine recent by HPLC-UV with RP -SPE extraction. **Materials and Methods:** HPLC Waters© was used with a 2498 UV Waters detector and Aminex HPX-87H (300 mm x 7.8 mm 9 uM) BIO-RAD column working in the isocratic mode. The mobile phase consist of $7{,}5x10^{-4}$ M sulfuric acid solution with a pH between 2.83 to 2.90. The flow of the mobile phase was 0.7 mL min^{-1}, with column temperature at 50 °C. The analysis time was 55 min and the detector operating at wavelength of 196 nm. The extraction performed using a reverse phase SPE cartridge Waters OASIS HLB 1 cc 30 mg. First, activates the cartridge eluting with 1.0 mL of methanol and then 1.0 mL deionized water. Add 0.5 mL of sample and collect in the test tube. Transfer the eluate to glass ampoule and sealing it. Keep the ampoule at 120 °C for 2h. Centrifuge at 10,000 rpm for 5 min. Transfer to a 2.0 mL vial and inject 20 uL into the chromatographic system. **Results:** The method is linear in the range from 2.0 to 52.0 $ng.mL^{-1}$. The method showed good intra- and inter-day precision, ranging from 2.2 to 5.8%, and accuracy of 90.4 to 101.9% recovery. The limits of detection and quantification obtained are 0.23 $ng.mL^{-1}$ and 0.74 $ng.mL^{-1}$, respectively. Comparison with other procedures also illustrates the performance of the method. When compared with GC-FID, was obtained correlation coefficient of 0.99. **Conclusion:** The procedure developed to measure NMF in urine samples using SPE cartridge in the reverse mode is simple and showed good precision and accuracy during validation testing. The procedure has been successfully applied in the evaluation of occupational exposure to Dimethylformamide.

OC 08- METABOLIC POLYMORPHISMS AND CLINICAL FINDINGS RELATED TO BENZENE POISONING IN BRAZILIAN EXPOSED GAS STATION WORKERS

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Introduction: Since benzene is present in gasoline and motor vehicle emissions, gas station workers have been occupationally exposed. Benzene exposure can lead to benzene poisoning (BP), and metabolic polymorphisms may alter the risk of benzene toxicity. **Objective**: In the current study, we evaluated alterations related to benzene poisoning and metabolic polymorphisms in gas station attendants exposed to benzene in Rio de Janeiro, Brazil. **Methodology**: We evaluated clinical findings related to BP, and metabolic polymorphisms in 114 Brazilian gas station attendants. The workers were divided into No Clinical Finding (NCF) and Clinical Findings (CF) groups. **Results and discussion**: We found alterations in 63.2%. CF group presented lower blood cell count (no significance). Neutrophil and MCV (indicative of macrocytosis) showed a significant difference between groups, and neutrophil has greatest impact on the alterations suggestive of BP. We found higher frequencies of symptoms in CF group, although not all presented statistical significance. The frequencies of risk alleles were higher in CF group for GSTM1, GSTT1, CYP2E1 7632T>A, but lower for NQO1 and CYP2E1 1053C>T genotypes. MPO variant allele showed no difference. We found an association between GSTM1 null and alterations related to BP, but we did not observe an effect of other polymorphisms. **Conclusion**: Further studies with larger sample size are needed to confirm these findings. Variations in benzene metabolizing genes may modify benzene toxicity and should be taken into consideration during the risk assessment evaluation.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OC 09- USES OF INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES) TO DETERMINE URANIUM IN URINE

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Introduction: The Uranium (U) occupational exposure poses risk to workers of the mines, arms industry, fertilizers and others (CDC, 2009). The U environmental concentrations are well established by the national and international regulatory agencies, however, there is no exposure limits consensus, as well as biological indicators of this exposure. **Aims:** Using the environmental guidelines (USEPA, 1994; OMS, 2000; PORT.2914, 2011) as a model, this study aiming to validate urine as an occupational exposure marker for U traces using the ICP-OES technique. **Material and Methods:** This study was performed with a Varian model 720-es ICP-OES with sequential detection. The analytical curve was range for 10.0 to 100.0 ug/L. The repeatability and analyte recovery were estimated by five replicates of fortified samples (urine) in three concentrations as follows; low (10.0 ug/L), intermediary (50.0 ug/L) and high (100.0 ug/L). **Results e Discussion:** The recovery ranged from 90 to 110% and the repeatability was lower than 10% in the 10.0 ug/L, 50.0 ug/L and 75.0 ug/L concentrations. The analytical curve was linear in the range studied (0,999 of correlation) and homoscedastic for the residues. The determination of U traces in urine showed satisfactory recovery and repeatability by ICP-OES according to the literature (ANVISA, 2003; INMETRO, 2011). **Conclusion:** The validated methodology can be used for monitoring U in the urine of subjects occupationally exposed.

References:

1- INMETRO. Documentos necessários para Acreditação de laboratórios de calibração e Ensaios segundo os requisitos da NBR ISO/IEC 17025. Disponível em http://goo.gl/cKBllw Acessado em 30/06/2015.
2 – ANVISA RESOLUÇÃO-RE Nº 899, DE 29 DE MAIO DE 2003. Guia para validação de métodos analíticos e bioanalíticos. Disponível em: http://goo.gl/ViYgDN. Acessado em 30/06/2015.
3- CDC. Agency for Toxic Substances and Disease Registry Case Studies in Environmental Medicine (CSEM). Disponível em http://www.atsdr.cdc.gov/csem/uranium/docs/uranium.pdf Acessado em 30/06/2015.
4 – U.S. EPA. Method 200.8 Determination of Trace Elements in Waters and Wastes by ICP-MS: Disponível em http://www.epa.gov/sam/pdfs/EPA-200.8.pdf Acessado em 30/06/2015.
5 - U. S. Environmental Protection Agency. Technical Fact Sheet: Final Rule for (Non-Radon) Radionuclides in Drinking Water.
6- BRASIL. Portaria nº 2914 de 12 de dezembro de 2011 do Ministério da Saúde.

OC 10- SURVEY OF PROFILE SOCIODEMOGRAPHIC AND USE PSYCHOACTIVE SUBSTANCE IN THE MILITARY POLICE THE STATE OF GOIÁS

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Introduction: Brazil is considered a medium consumption country of legal and illegal drugs , and alcohol, tobacco and marijuana the most consumed in the Brazilian population , followed by cocaine. The police lead a stressful work routine and are not free of the consumption of psychoactive substances. **Objective:** In order to know the reality of the military police of the state of Goiás, was rated the partial socio-demographic profile and the profile of consumption of psychoactive substances**. Materials and Methods:** This analysis corresponds to a cohort study of 373 volunteers police , interviewed by applying a questionnaire (ASSIST) in the Battalion Porangatu Military Police and the Hospital of the Military Police in the interior and in the state capital of Goias respectively , from October 2014 to February 2015**. Results and Discussion:** Of the 373 respondents , 352 police answered the questionnaire , of these 330 were males and 22 females . 60 % were between 40-50 years old and 84 % have completed high school , 68% overweight or obese and 77 % said they had used alcohol , followed by tobacco with 27% and marijuana 4 % over the last 3 months. Drug use by police is contrary to the activities performed by these professionals , as psychoactive substances produce changes in behavior , mood and cognition, as well as having great potential for abuse , leading to dependency. Consumption of such drugs can lead to distrust of professionalism and character of police , so it was observed that some volunteers had fear in completing the questionnaire act relating to the use of psychoactive substances , even having been guaranteed absolute confidentiality. **Conclusion:** Most of the State of Goiás Military Police respondents had between 41-50 years old , 84% completed high school and 68 % are overweight or are obese , and 77% have declared consumption of any psychoactive substance . Given this situation , it is clear that there is need to develop more research covering the police , related to the practice of proper nutrition , regular physical activity , as well as monitoring the consumption of licit and illicit drugs , so that can be made better monitoring of military force in the state of Goiás.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OC 11- SURVEY OF PROFILE SOCIODEMOGRAPHIC AND USE PSYCHOACTIVE SUBSTANCE IN THE MILITARY POLICE THE STATE OF GOIÁS

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Introduction: Brazil is considered a medium consumption country of legal and illegal drugs , and alcohol, tobacco and marijuana the most consumed in the Brazilian population , followed by cocaine. The police lead a stressful work routine and are not free of the consumption of psychoactive substances. **Objective:** In order to know the reality of the military police of the state of Goiás, was rated the partial socio-demographic profile and the profile of consumption of psychoactive substances**. Materials and Methods:** This analysis corresponds to a cohort study of 373 volunteers police , interviewed by applying a questionnaire (ASSIST) in the Battalion Porangatu Military Police and the Hospital of the Military Police in the interior and in the state capital of Goias respectively , from October 2014 to February 2015**. Results and Discussion:** Of the 373 respondents , 352 police answered the questionnaire , of these 330 were males and 22 females . 60 % were between 40-50 years old and 84 % have completed high school , 68% overweight or obese and 77 % said they had used alcohol , followed by tobacco with 27% and marijuana 4 % over the last 3 months. Drug use by police is contrary to the activities performed by these professionals , as psychoactive substances produce changes in behavior , mood and cognition, as well as having great potential for abuse , leading to dependency. Consumption of such drugs can lead to distrust of professionalism and character of police , so it was observed that some volunteers had fear in completing the questionnaire act relating to the use of psychoactive substances , even having been guaranteed absolute confidentiality. **Conclusion:** Most of the State of Goiás Military Police respondents had between 41-50 years old , 84% completed high school and 68 % are overweight or are obese , and 77% have declared consumption of any psychoactive substance . Given this situation , it is clear that there is need to develop more research covering the police , related to the practice of proper nutrition , regular physical activity , as well as monitoring the consumption of licit and illicit drugs , so that can be made better monitoring of military force in the state of Goiás.

OC 12- SURVEY OF PROFILE SOCIODEMOGRAPHIC AND USE PSYCHOACTIVE SUBSTANCE IN THE MILITARY POLICE THE STATE OF GOIÁS

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Introduction: Brazil is considered a medium consumption country of legal and illegal drugs , and alcohol, tobacco and marijuana the most consumed in the Brazilian population , followed by cocaine. The police lead a stressful work routine and are not free of the consumption of psychoactive substances. **Objective:** In order to know the reality of the military police of the state of Goiás, was rated the partial socio-demographic profile and the profile of consumption of psychoactive substances**. Materials and Methods:** This analysis corresponds to a cohort study of 373 volunteers police , interviewed by applying a questionnaire (ASSIST) in the Battalion Porangatu Military Police and the Hospital of the Military Police in the interior and in the state capital of Goias respectively , from October 2014 to February 2015**. Results and Discussion:** Of the 373 respondents , 352 police answered the questionnaire , of these 330 were males and 22 females . 60 % were between 40-50 years old and 84 % have completed high school , 68% overweight or obese and 77 % said they had used alcohol , followed by tobacco with 27% and marijuana 4 % over the last 3 months. Drug use by police is contrary to the activities performed by these professionals , as psychoactive substances produce changes in behavior , mood and cognition, as well as having great potential for abuse , leading to dependency. Consumption of such drugs can lead to distrust of professionalism and character of police , so it was observed that some volunteers had fear in completing the questionnaire act relating to the use of psychoactive substances , even having been guaranteed absolute confidentiality. **Conclusion:** Most of the State of Goiás Military Police respondents had between 41-50 years old , 84% completed high school and 68 % are overweight or are obese , and 77% have declared consumption of any psychoactive substance . Given this situation , it is clear that there is need to develop more research covering the police , related to the practice of proper nutrition , regular physical activity , as well as monitoring the consumption of licit and illicit drugs , so that can be made better monitoring of military force in the state of Goiás.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

OC 13- PESTICIDE EXPOSURE AND RESPIRATORY SYMPTOMS AMONG FARMERS IN SAO JOSE DE UBA, RIO DE JANEIRO STATE, BRAZIL.

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Introduction: Excessive use of pesticides in agriculture is a major risk to human health, especially to farm workers. The airway is one of the main routes of exposure and respiratory system can react with various symptoms and different intensities. The economy of Sao Jose de Uba, a small community located in Rio de Janeiro State is based on tomato's crop, which requires much pesticide due to its high perishability. **Objective:** To evaluate the presence of respiratory symptoms in farm workers exposed to pesticides during the periods of season and off-season in Sao Jose de Uba, Rio de Janeiro State. **Materials and Methods:** Study subjects were interviewed using questionnaires to access socioeconomic factors and confounders (such as smoking history) and to estimate pesticide exposure and prevalence of respiratory symptoms. 44 farmers (37 men and 7 women) were interviewed during the season (Aug/2014) and off-season (Jan/2015). The symptoms investigated were cough, dyspnea, tightness or wheezing, rhinitis or allergy in nose in the last 4 months to compare the prevalence between periods of higher and lower exposure. **Results and Discussion:** The mean age of farmers was 43.1 (range 18-74) years old and the mean exposure time was 31.4 years with a range of 5-66 exposure-years. Many participants were very young (mean 12.4 years old) when started working or helping in the crop. Pesticides were mainly applied twice a week in season by manual pumping and backpack tank, the average hours worked daily was 10.7 (range 7-14) and most of farmers do not use full protective equipment. Regarding smoking history, 59% were nonsmokers, 20.5% were current smokers and 20.5% were former smokers. The current study found in the off-season a decrease in the number of symptomatic individuals and the prevalence of all symptoms investigated. During the harvest, 65.9% reported at least one symptom, while in the off-season only 36.4% reported something. The prevalence (and PR - Prevalence Ratio) of cough crisis was 41% in season and 20.5% in off-season (PR=2). For chest tightness and allergy in the nose or rhinitis, the prevalence were 22.7% in season and 15.9% in off-season (PR= 1.44). The prevalence of wheezing was 15.9% in season and 9.1% in off-season (PR=1.78). For shortness of breath, the prevalence was 11.4% in season and 9.1% in off-season (PR=1.22). **Conclusions:** A higher prevalence of respiratory symptoms found among farm workers in the season compared with off-season shows the susceptibility of the respiratory system to acute poisoning by pesticides and stresses the importance of adoption of methods to reduce exposure and mitigate risks. Further approach should concentrate on exposure data and statistical analysis.

OC 14- EVALUATION OF PLASMA CHOLINESTERASE ACTIVITY, IN AGRICULTURAL WORKERS OF SIERRA TALHADA, PERNAMBUCO.

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INTRODUCTION: Chemical pesticides are considered toxic agents. The interaction of a toxic agent, such as pesticides, with the body causes various specific signs or symptoms and is called poisoning. There are three types of poisoning: acute, sub-chronic, and chronic. In acute poisoning symptoms appear quickly, at most a few hours after a short period of exposure to toxic agents. There may be mild, moderate or severe symptoms, depending on the amount of the substance absorbed by the body and the body's sensitivity. Sub-chronic poisoning occurs with light to moderate exposure to moderately or highly toxic agents, and has a slower onset. The symptoms are subjective and vague, and include headache, weakness, malaise, stomach pain, and drowsiness, among others. Chronic poisoning is characterized by a delayed onset of symptoms, often months to years after light to moderate exposure to one or multiple toxic agents. Chronic poisoning causes irreversible damage, such as paralysis or cancer, among other things, and may even lead to death. **OBJECTIVE:** Our work focused on assessing the exposure of workers to organophosphates and carbamates, as they are cholinesterase enzyme inhibitors. 256 agricultural workers from Serra Talhada, a small town located in the dry lands of Pernambuco, were analyzed during the period from January to December 2014. **MATERIALS AND METHODS**: The Ellman method was used in the analysis of plasma cholinesterase activity, through the Test-mate ChE, whichis a complete system for testing plasma and erythrocyte cholinesterase. **RESULTS**: We observed among exposed workers analyzed (n = 256), 25% (n-65), presented levels indicating inhibition of plasma cholinesterase. It was also noted that 27.69% (n = 18) had levels of plasma cholinesterase below 1.00 U / mL, suggesting a possible poisoning. **DISCUSSION**: The data obtained were related to signs and symptoms reported by workers in a questionnaire that was given. These symptoms included vomiting, headache, dizziness, nausea, breathing difficulties and other symptoms. Some of the subjects presented diseases such as diabetes, hypertension, arthritis, gastric distress, problems of the liver, and allergies. The time of exposure to pesticides ranged from 06 months to 30 years. Among the pesticides the workers were exposed to, the use of organochlorines, pyrethroids, organophosphates, and carbamates was verified. **CONCLUSION**: Within what was revealed, it was verified that there was significant inhibition of plasma cholinesterase in the group tested, reinforcing the need for the monitoring of workers as a matter of public health.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

OC 15- VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF S-PHENYLMERCAPTURIC ACID (S-PMA) IN URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUOROMETRIC DETECTOR

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Introduction: Benzene is an aromatic hydrocarbon, naturally present in petroleum and as by-product in the steel industry. Benzene is a important occupational and environmental contaminant due its toxicological significance, as a carcinogen. Then there are not limits to regulate a safe occupational exposure. Toxicological studies in animals and humans showed pronounced myelotoxic action, cause of leukemic disorders, blood cells disorders (Thomas et al., 2014). Assessing of benzene exposure is performed by biomarkers as *trans, trans*-muconic acid (*t,t* MA) and S-phenylmercapturic acid (S-PMA) in the urine. However, because of no specificity of *t,t* MA, S-PMA has been proposed to asses lower levels of exposure and as an specific biomarker of benzene. **Objective:** The aim of study is validate an analytical method for the quantification of S-PMA by high-performance liquid chromatography with fluorometric detector. **Methods:** The development of an analytical method was based on previous published studies (Einig and Dehnen, 1995; Buratti et al., 2001). The S-PMA present in the urine was extracted by solid phase extraction (SPE) using C-18 phase. The eluated were submited to water bath at 75 ° C and nitrogen to analyte concentration, followed by alkaline hydrolysis and derivatization with monobromobimane. On the chromatographic analysis it was used a reverse phase C-18 column with 240 mm length, 4 mm in diameter and 5 m particle; the mobile phase was acetonitrile and 0,5% acetic acid (50:50) with the flow of 0,8 mL/min and column temperature 35°C. **Results:** The limits of detection and quantification were 0.71µg/L and 2.2µg/L, respectively. The linearity was verified by simple linear regression, and the method exhibited good linearity in the range of 10-100 µg/L. There was no matrix effect for S-PMA using concentrations of 40, 60, 80 and 100 µg/L. The intra- and interassay precision showed coefficient of variation of less than 10% and the recovery ranged from 83.4% to 102.8% with an average of 94.4%. The stability of S-PMA urine stored at -20°C was of seven weeks. **Conclusion:** The figures of merit presented satisfactory results and the proposed method for determining urinary S-PMA showed adequate sensitivity for assessment of occupational and environmental exposure to benzene using S-PMA as exposure biomarker.

BIBLIOGRAPHIC REFERENCES

- Buratti, M. et al. Determination of monobromobimane derivatives of phenylmercapturic and benzylmercapturic acids in urine by high- performance liquid chromatography and fluorimetry. **J Chromat B**v. 751, n. 2, p. 305-313, 2001.
- Einig, T.; Dehnen, W. Sensitive determination of the benzene metabolite S- phenylmercapturic acid in urine by high- performance liquid chromatography with fluorescence detection. **J Chromat A,** v. 697, n. 1, p. 371-375, 1995.
- Thomas, R. et al. Characterization of Changes in Gene Expression and Biochemical Pathways at Low Levels of Benzene Exposure. **PLoS One,** v. 9, n. 5, 2014. ISSN 1932-6203.

RELATIONSHIP BETWEEN BUCCAL MICRONUCLEUS CYTOME ASSAY AND URINARY 1-HYDROXYPYRENE LEVELS AMONG CASHEW NUT ROASTING WORKERS.

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The artisanal cashew nut roasting is an important economic and social activity around the world. However, one of the main issues is the conditions under which cashew roasting takes place. The present study conducted the assessment of occupational risk of this activity by the use of biomarkers of exposure and effect, as well as the analysis of the released particulate matter (PM). To quantify $PM_{1.0}$, $PM_{2.5}$, PM_{10} and TPS concentrations a real-time airborne particle monitor was used. The morphology, particle size distribution and elemental composition was determined using scanning electron microscopy coupled with energy-dispersive x-ray spectrometry (SEM-EDS). Trajectories, dispersion and deposition of the emitted material were calculated using the NOAA-HYSPLIT model. Urinary 1-hydroxypyrene (1-OHP) levels were analyzed by high-performance liquid chromatography. DNA damage, chromosomal instability and cell death were measured by the buccal micronucleus cytome assay (BMCyt assay). The PM concentrations in the exposed area were higher than in the non-exposed area ($p < 0.0001$). Furthermore, it was observed that the control area yielded a higher prevalence of coarse particles, while in the exposed area a higher prevalence of fine particles was observed. The SEM-EDS analyses showed a wide variety of irregular particles in the samples, such as spherical, smooth-surfaced, cubic, mineral particles and aggregate particles. Biomass burning tracers K, Cl, S and Ca were the major inorganic compounds found. The dispersion analysis suggested that the $PM_{2.5}$ can reach neighboring regions in a distance in the order of 40 km. PAH exposure was confirmed by increases of 1-OHP levels in cashew nut workers. The frequency of micronuclei (MN), nuclear buds, binucleated cells, karyorrhexis, karyolytic and pyknotic cells were higher in the exposed group ($p < 0.0001$). The influence of factors such as age range, smoking status and family history of cancer on the MN frequency was evidenced and it was observed a correlation ($r = 0.61$; $p < 0.0001$) between the exposure (1-OHP) and effect (MN) biomarker. Our data shows that artisanal cashew nut roasting is a serious occupational issue, with harmful effects on workers' health. The PAH exposure and genotoxic potential among cashew nut workers were confirmed by the increase of urinary 1-OHP levels and BMCyt biomarkers, with positive significant correlation. The uses of exposure and effect biomarkers were therefore efficient to assess the occupational risk associated with artisanal cashew nut roasting. The high rates of $PM_{2.5}$ are considered a potential contributor to this effect and the use of portable real-time aerosol monitors in areas with difficult access are an alternative as an initial screening of PM measurements.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

MEDICINES AND COSMETICS TOXICOLOGY

A | R | T
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MD 01- SAFETY EVALUATION OF TECHNICALLY UNAVOIDABLE TRACES OF METALS IN COSMETICS

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According to EU Regulation no.1223/2009/CE cosmetic products for daily use can contain "technically unavoidable traces" of metals. This definition is too vague. Authorities should set well defined limits, considering the risks associated with metal contamination of personal care products (PCPs). This presentation characterizes the risk arising from a number of metals (antimony, arsenic, cadmium, cobalt, chromium, mercury, nickel, lead) that may occur in raw materials and, consequently, in PCPs. A worst case scenario is adopted, based on the following assumptions: i) the individual ingredients contained the maximum amount ever measured for each metal, ii) the hypothetical PCP was produced exclusively with that single ingredient; iii) when absorption through the skin was not known, data related to oral absorption were used. Risk characterization was performed calculating the Systemic Exposure Dosage (SED) and the Margin of Safety (MoS=NOAEL or BMDL10/SED). Exposure to the allegedly "technically unavoidable" maximum amounts of metals in cosmetic ingredients resulted in MoSs exceeding 100 (safety threshold) with one exception. This suggests that the availability of experimental dermal absorption rates could enable significant improvement in MoS, thus increasing safety levels. Although results are reassuring, the authors recommend minimization of contamination, according to the state of the art of manufacturing methods.

MD 02- TANNIN FRACTION EXTRACTED OF VINHÁTICO (*Plathymenia reticulata* BENTH 1842) CAN INDUCE DNA DAMAGE AS EVIDENCED BY UP EXPRESSION OF RNR3

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Introduction: In the Brazilian folk medicine *Plathymenia reticulata* Benth (Fabaceae), known as "vinhático" (wine-like), has been traditionally used to treat inflammatory processes, including important applications as antiophidic drug. In order to assess the security of new drugs derived from native plants, the governmental agency (Anvisa) recommend the adoption of mutagenic test in distinct biological levels (organism, cellular and molecular) as well as, distinct experimental systems (*in vitro* and *in vivo*). Rodent micronucleus test and analysis of differential gene expression in the model *Saccharomyces cerevisiae* are popular assays to evaluate mutagenic effect of plant drugs. **Objective:** Discriminate the phytochemical profile of aqueous extract of *P. reticulata* (EAPr) and test the mutagenic/genotoxic effects of tannin fraction purified of *P.reticulata* (TCPr). **Materials and Methods**: The phytochemical profile of EAPr was performed by Thin Layer Chromatography (TLC) and the TCPr was purified by column chromatography using silica gel Sephadex LH20. The mutagenic and genotoxic effect of TCPr were analyzed, *in vivo,* by Micronucleus Test (MN) and qRT-PCR method, respectively. For the MN Test, *Swiss* mice (5 groups, *n*=6 each) were orally treated with three different doses of TCPr (25, 50 and 500 mg kg^{-1}). A positive (50 mg kg^{-1} cyclophosphamide) and negative control group (10 mL kg^{-1} of distilled water) were included. All the treatments were evaluated 24 h after administration. To evaluate the genotoxic effect of TCPr at molecular level, cultures of *Saccharomyces cerevisiae* (Δycf1) were exposed to concentration of 125 µg mL^{-1} of this extract and the expression of RNR3 (Ribonucleotide reductase) gene, recognized for being induced in the presence of different types of damage DNA inducers, was analyzed by real-time qPCR. **Results and Discussion:** The phytochemical profile of EAPr showed the presence of thymol, rutin, aesculin, 1,2-benzopyrone, catechin and gallic acid and condensed tannins, whose were purified by column chromatography. The MN test not showed mutagenic effect in bone marrow cells of mice. On the other hand, the gene expression profiles, when compared with that of untreated cultures, showed that TCPr promoted significant gene induction, increased 32-fold the *RNR3* gene expression. **Conclusion:** The tannin fraction purified of *P. reticulata* potentially can induce DNA damage as demonstrated with the increased *RNR3* gene expression, indicating a genotoxic effect probably linked to the onset of an oxidative stress pathway.

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A | R | T
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MD 03- ADVERSE EFFECTS OF LOCAL ANESTHETICS USED IN DENTISTRY: REVIEW

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Introduction: Local anesthetics cause temporary blockage of the conduction of neural impulses. In practice, the advantage of local anesthetics is that their action is fully reversible, while causing loss of feeling and no change in level of consciousness. After finishing the anesthetic effect, there is a complete recovery of nervous function without evidencing structural damage to the cells or in the nerve fibers. However, when administrated in high doses, or in the even to accidental intravascular punctures some adverse reactions are reported. **Goals:** This study has the objective of verifying which the adverse effects related to local anesthetics solutions used in dentistry. **Materials and methods:** This study deals with a partially systematic review. The search for the articles washeld in BIREME and PUBMED databases with the followingin tersections: dentistry, local anesthetics, adverse effects and dentistry and anesthetics. **Results and discussion:** The use of anesthetics solutions raises many questions to dentists, as to its applicability, as to the toxicity of the different solutions available on the market. Among the preference of the professionals are: lidocaine, prilocaine, mepivacaine and bupivacaine. In addition to these, others are employed as articaine, ropivacaine and levobupivacaine, and these last two are safer alternatives to bupivacaine, due to lower systemic toxicity. Once absorbed in to the bloodstream, local anesthetics are distributed to all tissues of the body, having a half-life ranging from minutes to hours, depending upon the drug employed. The local anesthetic metabolism is important because the overall toxicity of the drug depends on the balance between the rate of blood absorption at the injection site and the speed at which it is removed from the blood through the tissue absorption and metabolism processes. The complications caused by these local anesthetic can be divided into non-psychogenic and psychogenic. The first depend on the anesthetic and are related to the stress state of the patient. The most common occurrences are fainting, and hyperventilation. The non-psychogenic complication are rare and related to the improper technique of administration, overdose or allergic reaction to the anesthetic. **Conclusions:** Considering the information available, it is concluded that the anesthetic agent should have low systemic toxicity, be non-irritating to the tissues and do not cause permanent damage to the nervous structures. The onset time of the anesthetic effect should be as short as possible and the duration of action sufficient for the surgical procedure, with reversible action, besides respecting to the clinical indications for each type of anesthetic solution in order to optimize its potential, thus valuing the welfare of the patient.

MD 04- LONG-TERM REPRODUCTIVE IMPAIRMENT IN MALE RATS EXPOSED PRENATALLY TO THE GLUCOCORTICOID BETAMETHASONE

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Introduction: Betamethasone (BM) is the drug of choice for antenatal treatment, promoting fetal lung maturation, decreasing the incidence of respiratory distress syndrome and neonatal mortality. Previous studies reported that prenatal BM treatment reduced testosterone levels and impaired sperm quality and fertility in adult rats. **Objective:** To further evaluate the reproductive consequences of prenatal BM exposure in male rats. **Material and methods:** Pregnant Wistar rats (n=10/group) were separated into two groups: control (vehicle) and BM-treated (0.1mg/kg IM). Rats were injected on gestational days 12, 13, 18 and 19 when germ cell migration and masculinization occurs. Body weight was measured at postnatal day (PND) 1 and 90. One male from each litter was used to evaluate sexual behavior performance and fertility. Rats were killed 30 days later and the following parameters were evaluated: body and reproductive organ weights, serum hormone levels, sperm parameters, and fertility after *in utero* artificial insemination. Another subset of males was selected to assess accessory gland contractility, testicular morphology, and localization of the gap junction protein Connexin 43 (Cx43) and proliferating cell nuclear antigen (PCNA). **Results and discussion:** There was a significant reduction in body weight at PND 1, and sperm motility and production in adults of the BM-treated rats. Furthermore, seminal vesicle weights were decreased while testicular and ventral prostate weights were increased. Serum LH levels and the percentage of abnormal sperm were significantly increased by BM. Interestingly, a higher percentage of seminiferous tubules displayed abnormal morphology (95% in the control vs 80% in the treated group). Many tubules displayed disrupted germ cell organization and distribution. These showed abnormal migration of Sertoli and germ cells toward the lumen of the seminiferous tubule. Immunostaining of Cx43 was less intense in the treated group. In tubules with altered cell migration, Cx43 immunostaining was apical but remained associated with the mis-localized Sertoli cells. Furthermore, PCNA localization revealed the presence of spermatogonia associated with these mis-localized Sertoli cells, thus confirming the abnormal migration of Sertoli and germ cells. Although sexual behavior was not altered, a significant reduction in fertility in the adult rats exposed prenatally to BM was noted. **Conclusion:** Prenatal BM exposure leads to long-term reproductive impairment in male rats. Histopathological analyses revealed uncommon testicular lesions in adults and diminished fertility. These results may have important implications for humans, considering the use of BM in pregnant women.

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A | R | T
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MD 05- DRUG INTOXICATION IN THE STATE OF PARAÍBA

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Introduction: The drugs have an important role in restoring and maintaining health, and is considered the most common form of therapy in our society. However, there are studies that demonstrate the existence of various diseases related to medication, among them drug intoxication, reaching thousands of people around the world today. Countless are the causes of poisoning, including, self-medication, accidental use and problems related to own habitual use has been observed in such cases. In Brazil, drugs take first place in SINITOX statistics (National System of Toxic and Pharmacological Poison) as the primary toxic poisoning-causing agent in humans since 1994. The improper and indiscriminate use of drugs contributes to the increase in cases and consequently the number of deaths. In this context, it was noted paucity of research related to this topic in the Paraíba region, which led to the preparation of this work. **Objectives**: To describe the epidemiological profile of individuals reported with drug intoxication in the state of Paraiba from January 2012 to February 2015. **Designs and methods:** This is a study of cross-field retrospective, with qualitative and quantitative approach. We collected data on drug poisonings reported to SINAN, the health authorities of the state of Paraíba, in the period from January 2012 to February 2015. **Results and discussion**: In the period 2012-2015, there were 1,868 cases of such poisoning reported in the state of Paraíba. Among those items considered by SINAN, around 30% of cases intoxication was consummated by use of drugs to attempt suicide, a fact that is due to large toxic property of some medications, so it is a way for depressed individuals to self-violence. Other cases as self-medication, accidental use and the actual therapeutic use have been reported. Regarding age, it was observed that 40% of cases ulcers were people who had 20-34 years because this individual more prone to stress conditions resulting from responsibility in adulthood. About sex 64% of cases were female, as related to the fact that women use more drugs than men because of hormonal situations and the search for aesthetics through some medications. Only 0.3% progressed to death, confirming the quality of care and therapeutic efficiency, affiliating these cases are referred on an emergency basis to hospital areas to attempt to reverse the poisoning. **Conclusion:** Due to the profile of users are suggested preventive actions that aim to prepare them psychologically to deal with stressful situations of everyday life, and promotion of information related to the proper use of medications, possible interactions and proper storage to each situation. The results obtained indicated a service quality used in the therapy and cure as evidenced in most cases.

MD 06- PROFILE OF INTOXICATION BY DRUG REGISTERED IN SOUSA-PB TOWN

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Introduction: The intoxication is characterized as a clinical manifestation of harmful effects produced in a living organism as a result of the interaction of a toxic agent that body. Several authors cite the drug as one of the main agents responsible for poisoning. To develop better treatment, it is necessary to monitor these poisonings by drugs, which is done on a mandatory basis by health authorities through a database called SINAN (Diseases Information System Notification). In this context, it was noted paucity of research related to this theme in Sousa-PB region, which led to the preparation of this work. **Objectives:** Describe the epidemiology of intoxication by drugs in 15 cities attended the 10th GSP (Paraíba State Health Management) in the period from 2011 to 2014. **Designs and methods:** This is a study of cross-sectional retrospective field, with qualitative and quantitative approach aimed to evaluate epidemiological data collected in SINAN relating to poisoning by drugs notified by the health authorities in 15 municipalities attended the 10th GSP, between January 2011 to December 2014. **Results and discussion:** There have been reports of poisoning 45 drugs cases. A similar studies it was found that the most affected population was female with 64% of cases. A similar result was found in a survey conducted in the State of Goiás (Rios et al., 2005), in which the authors justify this as being intrinsic to brazilian culture, as the women attempt suicide more than men. According age group, the young of 16 to 25yrs were the most affected featuring 44% .According to the study by Bernardes and Schmidt, young adults, aged 20 and 29, tend to self-medicate, mainly by total livre. Do prescription drugs cases analyzed, there were 29 drugs distributed in 13 therapeutic classes. The benzodiazepines (BDZs) accounted for 16% of poisonings registered. These data may be related to the fact that some general practitioners are large prescribers of BDZs. The analysis of therapeutic classes on key circumstances shows expressive use of BDZs in suicide attempts, individual accidents and in cases of self-medication. **Conclusion:** It was found that drug intoxication and self-medication are interconnected, drug classes whose sale requires presentation and / or retention of the prescription, warning of the need for sharper measures involving increased awareness and monitoring of the professionals involved in process of prescribing and dispensing.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MD 07- TWO YEARS OF COSMETOVIGILANCE DATA ON FACE PRODUCTS: IS ACNE A RELEVANT ADVERSE OUTCOME FOR COSMETICS?

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Introduction: Cosmetics are very popular and once it is considered safe, their use continues to increase. However, during recent decades, we have become aware that adverse effects can occur. According to the Brazilian Society of Dermatology, acne is the main reason why Brazilians visit the dermatologist. This disease affects all to a certain degree and induces significant psychosocial consequences. However, there are few data about its epidemiology and how products can influence this condition. A structured vigilance system is a valuable tool for monitoring the safety profile of products, for information purposes and for product improvement, as well as meeting the requirements of health authorities and consumers. **Objective**: The objective was to determine the consumer characteristics and whether there was any time effect or product type effect. **Materials and Methods**: 66 face products (makeup foundation and powder, sunscreen and face treatment products) were chosen to access the characteristics of the population that complains about acne after using cosmetics. Cosmetovigilance reports from November 2010 to August 2012 were analyzed. Only spontaneous reports of Brazilian consumers related to acne were considered. The data related to product category was based on notification rate (number of complaints received per unit of product sold). Other parameters were based on percentage of reports. Descriptive statistics and exploratory analysis were done using the software XLSTAT, 2012. The confidence interval of 95% was used. **Results and Discussion**: The number of reported adverse reactions is very low, probably because of underreporting. According to our results, adverse effects occur in the first month of product use, during the first applications. The consumer is totally recovered in a short period after discontinuing it. Women (98%) complain more than men. 39% of the complaints belong to people from 26 to 35 years old, while 25% represents people from 36 to 45 years old. 18% of the consumers that complain are older than 45 years old and 7% are younger than 25 years old. There are more complaints from the Southeast Region of Brazil (73.7%), followed by the South (14.7%), Northeast (5.5%), Midwest (5.3%) and North (1%). The product category that has more complaints is face care (Notification rate – 0.024%), followed by makeup products (0.015%) and sunscreens (0.002%). It could happen because exposure to makeup and sunscreen is punctual and usually concomitant with other face products. In contrast, face treatment products are mostly used daily, which means a greater exposure. **Conclusion**: Despite the low amount of complaints from consumers that experience acne after using cosmetics, it is possible to get valuable information in order to provide better products.

MD 08- EFFECT OF QUERCETIN IN HYPERGLYCEMIA INDUCED BY TAMOXIFEN

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Introduction: Tamoxifen was discovered in 1970 and classified as a selective estrogen receptor modulator (SERM), since then it has been used as therapy in the treatment of breast cancer in post menopausal women. Although it has proven benefits, tamoxifen has caused side effects related to different mechanisms of toxic action, such as lipid peroxidation, oxidative stress and changes in the glycolytic pathway that induces hyperglycemia. Studies suggest that co-administration of quercetin, a flavonoid distribuited on onions, apples and grapes, with antioxidant potential, helps reduce side effects caused by tamoxifen. **Objective:** Investigate the effect of quercetin in hyperglycemia induced by tamoxifen. **Materials and methods:** For the study, healthy rats were ovariectomized (to simulate the post menopausal period) and treated orally for 14 days. The rats were divided in 6 groups: one control group with ovariectomized rats (OVX group), a group with ovariectomized rats treated with tamoxifen 5 $mg.kg^{-1}$ (TAM group), and four groups treated with tamoxifen 5 $mg.kg^{-1}$ and different concentrations of quercetin (2.5 $mg.kg^{-1}$; 7.5 $mg.kg^{-1}$; 22.5 $mg.kg^{-1}$ and 67.5 $mg.kg^{-1}$), called respectively, TAM+$Q_{2.5}$, TAM+$Q_{7.5}$, TAM+$Q_{22.5}$ and TAM+$Q_{67.5}$. Biochemical parameters, plasma glucose and hepatic glycogen levels were measured. The results were considered statistically significant when $p < 0.05$. **Results and discussion:** A significant increase of 75% in glucose levels and an increase of 204% in hepatic glycogen was observed on TAM group compared to the OVX group ($p<0.05$) confirming the hyperglycemic effect generated by tamoxifen. The analysis showed a significant change in glucose levels between the groups treated with tamoxifen and quercetin in different concentrations ($p<0.05$), generally occurring a decrease in glucose levels of groups TAM+$Q_{2.5}$ (197.63$mg.dL^{-1}$), TAM+$Q_{7.5}$ (198.97 $mg.dL^{-1}$), TAM+$Q_{22.5}$ (130.73 $mg.dL^{-1}$) and TAM+$Q_{67.5}$ (193.48 $mg.dL^{-1}$) compared to the TAM group (225.27 $mg.dL^{-1}$). However, the decrease in glucose levels does not result in a consequent significant variation of the hepatic glycogen ($p>0.05$). The group treated with quercetin at a concentration of 22.5 $mg.kg^{-1}$, showed the most effective hypoglycemic action against the hyperglycemic effect of tamoxifen, suggesting that at this concentration, quercetin is capable of antagonizing hyperglycemia caused by tamoxifen. Possibly, the antagonistic action of quercetin is due to the influence on tamoxifen bioavailability associated with the antioxidant properties of quercetin in tissues, restoring the damage caused by tamoxifen and so the homeostasis in the glycolytic pathway. **Conclusions:** The results suggest that the quercetin have antagonist potential action in the hyperglycemic effect caused by tamoxifen.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MD 09- KIDNEY PARAMATERS IN MALE RATS TREATED INTRAPERITONEALLY WITH ANTIOPHIDIC (ESPECÍFICO PESSÔA) EXTRACT

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Introduction: For more than 30 years this hydroalcoholic extract of the root of plant "cabeça-de-negro" has been used as supportive therapy in envenoming by snakebites in different regions of Brazil. Studies show that the antiophidic action is related to the two pterocarpans, cabenegrin A-I and A-II. Despite its widespread use there is little or no information in the literature about this extract. This lack of information and the wide use of this extract led us to investigate the effect, at the recommended dose, of the antiophidic extract Específico Pessôa on healthy animals and understand about its safety/toxic effect. **Objective:** Investigate the kidney effect of the antiophidic Específico Pessôa extract. **Materials and methods:** For the study, healthy rats were treated intraperitoneally with a single dose of 0.25 $mL.kg^{-1}$ with antiophidic Específico Pessôa extract (called EP group) and on the 15th day the animals were sacrificed. To check the influence of alcohol present in the extract on the experimental design, a respective alcohol group was added. Finally, a control group with healthy animals not treated was added. Kidney parameters (creatinine, urea and uric acid) were measured and the kidney was subjected to histopathological analysis. The results were considered statistically significant when $p < 0.05$. **Results and discussion:** The Antiophidic Específico Pessôa extract significantly affect renal parameters, with an increase by 17%, 33% and 206% in creatinine, urea and uric acid levels, respectively, compared to the control group ($p<0.05$), but not significative when compared to alcohol group ($p>0.05$). The action of alcohol alone on the biological system is relatively complex and the interaction between pterocarpan and alcohol is unknown. The results show that the alterations observed can be a result of an effect of antiophidic extract, particularly the pterocarpan, since the animals treated with alcohol alone not showed the same alterations when compared to control group ($p>0.05$). However the results demonstred that the alterations in renal system can be accentuated due to the interaction of the pterocarpans with alcohol contained in the antiophidic extract taking into account that the changes in the EP group with compared to alcohol group are not significant ($p>0.05$). Therefore, the increase in kidney paramaters in EP groups indicates initial kidney damage seeing that the histopathological analysis of the kidney did not show structural alterations. **Conclusions:** The analysis showed that the antiophidic Específico Pessôa extract interferes with kidney function and the changes observed can be a result of an independent effect of pterocarpan or of its interaction with alcohol.

MD 10- PRO-ADHESION EDUCATIONAL INTERVENTION MODEL FOR CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB IN GOIANIA, GOIÁS

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Introduction: The treatment of chronic myeloid leukemia (CML) changed dramatically with imatinib mesylate (IM). Besides the convenience of oral use, other benefits were achieved with the new drug, with faster therapeutic responses and increased survival, giving the CML similar characteristics as chronic diseases. Otherwise, there was another challenge, drug compliance, since a significant proportion of patients fail to ingest all the prescribed doses of IM. The concern was to optimize the adherence of CML patients, the hematology ambulatory (HC-UFG), led the authors to create a film cartoon, as a pro-adhesion educational intervention model. **Objective:** To investigate the effectiveness of this new educational material, in CML patients through direct and indirect assessment of adherence. **Materials and methods:** we used in 65 patients three adherence measures, for the indirect evaluation to used Morisky Test and Molecular Response (MR) and direct through therapeutic IM monitoring in plasma, before and after the screening of film. **Results and discussion:** In univariate analysis, from the Morisky Test, the film was striking, with increased adherent patients, which increased from 23.1% to 66.1%. The results of MR showed an improvement trend after the movie, because the positive molecular response (major MR or complete MR) increased from 81.5% to 86.1%. Regarding the serum levels of IM, with daily doses of 400-800 mg IM, the pre- movie samples showed higher average than the post-movie (2473.16 ± 1049.55 ng/ml versus 1414.72 ± 715 73 ng/ml), with a CV% interpatients of 43.4% and 50.6%, respectively. This high dispersion index found has been reported by other authors. On multivariate analysis, patients were divided into three groups: The first consists of women, 53 years old on average with associated diseases, before and after treatment of CML that use more than two drugs in addition to adhering IM before and after the film with a good therapeutic response. The second was marked by the change in non-adherence to pre-accession and post-film. Its characteristics were younger or fewer 53 years old, before absence of other diseases of CML, the use of less than two drugs and complete molecular response after the film. In the third, they are patients without molecular response before and after the educational intervention and non-adherence to IM after the film. They have age not exceeding 53 years old, and drug discontinuation due to adverse reactions, and resistant the educational film. **Conclusion:** medication adherence was higher among patients older than 53 years old, the educational film is an effective pro-adhesion assistance and continuing education, if combined with another method, it could help to maintain or enhance the benefits achieved in this work.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MD 11- ACUTE TOXICITY EVALUATION OF STANDARDIZED BRAZILIAN NORTHEAST MEDICINAL PLANT EXTRACTS

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Introdution: *L. tomentosa* Benth. Fritsch, *L. rigida* Benth., *C. impressa* Prance, *Spondias mombin* × *Spondias tuberosa* and *Turnera ulmifolia* Linn. var. *elegans* leaves extracts have been used as a traditional remedy for a wide range of ailments in humans. As part of the safety assessment of the extract, acute toxicity tests were conducted by the oral route in rats. **Objective:** Thus, in order to observe possible signs of toxicity and estimate the LD50, the aim of this study was to evaluate the acute toxicity of standardized extracts of medicinal plants of Northeast Brazil. **Material and methods:** The extracts were standardized by total phenolic content and HPLC profile analysis. Male and female rats were divided into 22 groups consisting of three rats each sex per group and given doses of 300–2000 mg/kg bwt. After the doses administration, compartmental parameters were observed. At the end of the experiment, the animals were sacrificed and blood and organs were collected to hematological, biochemical and histological analysis. The hematological parameters analyzed were total count of red blood cells, hemoglobin, hematocrit, mean corpuscular volume, average concentration of hemoglobin , mean corpuscular hemoglobin concentration, and total counts and differential leukocytes. As markers of liver function were assessed total protein, albumin, globulin and enzymes ALT, AST, γ-GT and bilirubin. Creatinine, urea were determined for renal evaluation. Total cholesterol, triglycerides, blood glucose and amylase were also analyzed. All experiments were ethical committee approved. **Result and discussion:** The extracts were standardized in accordance with the validation of bioanalytical methods standards recommended by ICH. And, the contents of total phenolics and the concentration of the major compound present in the extracts demonstrated high presence of phenolic compound, principality flavonol-3-*O*-glycosilated. During the experiment, no deaths were observed in any groups and there were no remarkable changes in general appearance, as well as in food and water consumption. No significant changes were noted in body and organs weights, hematological and serum biochemical parameters between the control and treated groups. **Conclusion:** Based on these findings, it can be inferred that the plants have no potential toxicity at certain dose levels; however cautions have to be taken when using of those plants for medicinal purposes. Thus, the acute use of the popular medicine extracts evaluated can be considered safe, which, added to the fact that their pharmacological effects are already being proven, studied species excellent candidates for the development of herbal future.

MD 12- STUDY OF SELF-MEDICATION PARACETAMOL AND ITS CORRELATION WITH DRUG INTOXICATION IN SINOP MUNICIPALITY.

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Background. Self-medication is a very common practice worldwide. Among the therapeutic classes of drugs, analgesics and antipyretics commonly used, with emphasis on paracetamol or N-acetyl-p-aminophenol, also known as acetaminophen. This drug induced hepatic injury in human and in experimental animals. Liver injury occurs due to binding its toxic metabolite, N-acetyl-p-benzoquinone, with protein and endogenous antioxidants. **Aim.** This study aimed to identify epidemiologically self-medication paracetamol in Sinop and to verify if this population has notions about the toxicity caused to the organism resulting from the frequent use of this drug. **Methods.** A field research was conducted from September to October of 2014, in which 100 customers in two drugstores in Sinop, Mato Grosso (MT), Brazil, were interviewed. The questionnaire comprised 11 objective and subjective questions on the self-medication. Participated in the study customers of both sexes, agreed to answer the questionnaire. All customers agreed to participate in the study and informed consent was obtained. The exclusion criteria included a non-agreement in answering the questionnaire, the lower customer under 18, and those who present insanity, drunkenness and errors when answering the questionnaire. **Results and Discussion.** The data were separated by participant age range, gender and the level of education. Of the 100 participants 94% reported practicing self-medication using analgesic and antipyretic. 35% of participants had a preference for paracetamol and 65% self-medicated with dipyrone. When the participants in the research were asked if paracetamol could cause damage to health as intoxication, only 38% reported believing that it can cause health problems. According to the results the primary causes that involve self-medication is the ease of purchasing drugs, as well as the overcrowding in the public hospitals due to the lack of medical and health professionals. There is also the self-confidence on the part of population to self-medicate, because most of the time, as the drug is freely available, the population considers these safe. The failure to report is also a major problem, know little about drug intoxication that can lead, from small hepatic changes to necrotic lesions in the liver and probably irreversible and can progress to death. **Conclusions**. Thus, we can conclude that in Sinop municipality there is a high index of people doing self-medication, in which the analgesic paracetamol is second choice for common symptoms, this drug can develop liver toxicity when used incorrectly. Therefore, its use should not occur randomly, so a better government policy to report on possible intoxication the population is required.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MD 13- COMPARISON OF THE PYROGEN *IN VIVO* METHODS DESCRIBED IN BRAZILIAN AND EUROPEAN PHARMACOPOEIAS: INTERFERENCE IN THE INTERPRETATION OF RESULTS

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Introduction: All injectable products for human use must be free of pyrogen. The *in vivo* pyrogen test is recommended in various pharmacopoeias. It's based on the fever reaction of rabbits. The Brazilian Pharmacopoeia follows the same method of the American, but differs the European in the number of rabbits, the number of steps in the test and the interpretation of results. **Objective:** The aim of this study was to compare both methods and identify if these differences can affect the final result of analysis, evaluating which was the more stringent criterion. **Materials and Methods:** The agreement and/or disagreement between the results of pharmacopoeias were analyzed by: a) 44 samples from Pyrogen Sector of Department of Pharmacology and Toxicology/INCQS that needed repetition of the assay. b) hypothetical data using the threshold value of fever of 0.5°C and 451 combinations by numerical modeling in a computer program simulating the first test. **Results and Discussion:** For all animals with individual temperature rise equal to 0.5°C, the Brazilian Pharmacopoeia had the result "pyrogen" for a sample whereas the European had the result "pyrogen free", demonstrating that the European Pharmacopoeia does not consider the elevation of 0.5°C as an indicator of fever. Both the routine data and the mathematically generated data showed that the Brazilian Pharmacopoeia was more rigid at low temperature rise (up to the sum of 1.15°C). Between the sum 1.2 ° C and 2.6 °C the pharmacopoeias had the same result forwarding the product to "go to next stage". The European Pharmacopoeia was stricter in high temperature rise (sum of above 2.7°C). **Conclusions:** The results indicate that the methodological differences may generate uncertainty in the evaluations of the protocols according to the country where the product was manufactured and may interfere in the interpretation of results.

MD 14- INVESTIGATION OF THE GENOTOXIC POTENTIAL OF A NEW BRAZILIAN BOTANICAL EXTRACT – A CASE STUDY

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A new botanical extract, obtained from the Brazilian plant *Casearia silvestris* was developed with the purpose of application in cosmetics. Following a standard approach for development of new cosmetic ingredients, the safety assessment was performed through a huge chemical and physical characterization and through a battery of alternative in vitro tests. To present and discuss the strategy defined to investigate an unexpected positive result obtained in the mammalian cell in vitro micronucleus test – OECD Test Guideline 487 - during the safety evaluation of the extract. As many other in vitro tests performed in monolayer cell cultures, the micronucleus test is criticized by its high rate of "misleading" positive results. The decision making in this situation became very challenger after the animal testing ban in Europe and a weight of evidence approach is needed to avoid unnecessary prohibition or disapproval of new ingredients. In the present case, the first step in the toxicological investigation was a critical assessment of chemical composition of the plant extract. With this information, a judgment of the plausibility of positive response in a genotoxicity test could be made. A literature review was performed for each molecule identified in the raw material. The most important finding was that the silica, which was present in the extract in concentration of 20%, could cause chromosomal aberrations and/or aneuploidies in V79 cells. After testing the aqueous extract without silica and the silica alone, the hypothesis of interference and misleading positive result was confirmed. The negative result in the raw material without silica was considered enough to support a favorable conclusion regarding genotoxicity. If no conclusion could be drawn from composition assessment, another step would still be needed, probably involving a more sophisticated test system, such as the reconstructed human skin, for example. This case provides an important learning about the interpretation of in vitro tests results and decision making process in the safety assessment of cosmetic ingredients.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MD 15- AGGREGATE EXPOSURE MODELS TO ESTIMATE THE EXPOSURE OF CONSUMERS TO FRAGRANCE INGREDIENTS IN COSMETIC PRODUCTS

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Introduction: Risk assessment of each ingredient is an important step during cosmetics products development to ensure the safe use for consumers and also meets all regulatory requirements. Some cosmetics could have more than 40 different ingredients besides the fragrances, which are mixtures of dozens of ingredients, some of them with allergenic potential. Use of several personal care products are part of the daily routine. Actual models to evaluate product safety consider a scenario that evaluate the hazard of all ingredients presents on certain formula, the target public and the mode of use, however this model do not consider that some ingredients could be also present in multiple products types presenting an aggregate exposure. This requires a more real life estimation of consumer exposure, including understand their habits in terms of frequency of use, areas of exposure and how much of each product is used daily. **Objective:** To develop models and predictions algorithms that take into account the aggregate exposure to fragrance ingredients presents on different cosmetic products used by Brazilian women, in order to perform a more realistic risk assessment of those products. **Materials and Methods:** 602 women aged 25-55 years residing in the cities of São Paulo, Recife and Porto Alegre answered a questioner made by Provoker Institute regarding their habits of using face cosmetic products. The aggregate exposure model was building based on a the following data: frequency of product use daily, amount per use, concentration of fragrance per product, retention factor, penetration factor, exposure area, body weight and height. Monte Carlo analyses were made to evaluate the more relevant variables that should be included and algorithms were used combining those data**. Results and Discussion:** Preliminary data have shown that this model can guide safety assessment of face cosmetic products considering more than one scenario of consumer habits of use (heavy user's women x minimalist users), their age, and ingredient concentrations to guide better product designers on a more realistic way. This is due to optimization of the model to establish relationships between the level of aggregate exposure and the likelihood of an adverse event, considering the exposure scenario of Brazil women population. **Conclusion:** Aggregate exposure models will be more and more used on risk assessment of ingredients by several industries sectors, since they have the advantage to combine different variables to simulate a more realistic exposure scenario, bringing more safety for costumers and less concern for regulators.

MD 16- DETERMINATION OF HEAVY METALS IN LIPSTICKS SOLD IN BRAZIL

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Introduction: Lipstick is a cosmetic widely used with the purpose of giving color to the lips. As cosmetics, lipsticks have several components in their formulation, including pigments and inorganic materials, which may contain toxic elements as natural constituents or as process contaminants. Metals such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) and mercury (Hg), when present in the composition, can be absorbed by oral or dermal routes, offering a risk to human health. However, there are no safety standards in Brazil regulating the metal levels in lipsticks. **Objective:** To determine the levels of five heavy metals, Pb, Cd, Cr, Ni and Hg in samples of lipsticks comercialized in Brazil, providing information for risk assessment of the use of these products for the consumer population. **Materials and Methods:** Fifty samples of lipsticks were acquired in the local market. About 100 mg of each sample were weighed on an analytical balance and subjected to microwave-assisted acid digestion. The quantification of metals was carried out by atomic absorption spectrometry in a graphite furnace (AAS-FG) to the elements: Ni, Cr, Pb and Cd and atomic absorption spectrometry with cold vapor generator for Hg. All samples were analyzed in duplicate. The accuracy was evaluated by recovery test. Precision was evaluated in terms of standard deviation. Because there is no reference material for the matrix analyzed, certified reference material from other matrices were used for quality control purpose. **Results and Discussion:** The analytical method presented adequate recovery and precision for the analysis of all elements. Pb, Cr and Ni metals were quantified in higher concentrations averaging 1.07 ± 1.251 µg/g, 1.04 ± 1.269 µg/g, 0.81 ± 0.964 µg/g, respectively. Cd and Hg were observed in low levels, only 3 of the samples had Hg levels above the detection limit. Weakly positive correlations were observed between Cr and Pb ($r=0.153$, $p<0.01$) and Cr and Ni ($r=0.296$, $p<0.01$). There was no influence of price or color of samples in the metals' concentrations. According to the origin, samples from China had significantly higher average content of Pb concentration than the others (China: 3.13 µg/g; Brazil: 0.95 µg/g; Taiwan: 0.26 µg/g e EUA: 0.32 µg/g). **Conclusion:** Although the levels detected suggest these products are low sources of exposure to metals, it should be considered that the daily use for a long period, along with other routes of exposure, could lead to accumulation in the organism and augmenting the likelihood of deleterious human health.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

MD 17- REGULATED NECROSIS TRIGGERED BY LQFM018: TOXICO-PHARMALOGICAL EVALUATION OF PIPERAZINE-CONTAINING COMPOUND

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Introduction: Recently necroptosis, a programmed cell death, has been identified as a non-apoptotic backup cell death mechanism with necrotic morphology. Piperazine derivatives have been widely used in biological screenings, providing their use in numerous applications, so that this scaffold is considered attractive for drug development. These heterocyclic molecules can act on several pharmacological targets, being found in a large variety of biological compounds developed to provide anticancer therapy. Preliminary studies performed by our laboratory have demonstrated that the piperazinic compound LQFM018, obtained from molecular simplification of LASSBio 579, an antipsychotic prototype, showed antiproliferative activity against cancer cells. **Objectives:** Therefore, in the present study we investigated the mechanisms of cytotoxicity, *immunosuppressive potential* and the acute oral toxicity of LQFM018. **Materials and Methods:** K562 cells were treated with LQFM018 and cytotoxic activities and the death molecular mechanisms were evaluated. Cell survival and phosphatidylserine externalization (PS) were assessed by trypan blue exclusion, MTT and flow cytometry using propidium iodide (PI) and anti-annexin-V antibodies. Morphological changes were evaluated using Hoechst 33342 and May-Grünwald-Giemsa dyes. Cell cycle analysis, mitochondrial membrane potential (ΔΨm), ROS, TNFR1, cytochrome-c and caspase activities were evaluated by flow cytometry. NF-kB, CYLD, caspase-3 and -8 mRNA levels were assessed by real time PCR. The effects of the compound were also investigated on 3D colony forming unit-granulocyte/macrophage (CFU-GM) and 3T3 basal cells. Acute oral toxicity was also conducted in mice (OECD 423). **Results and Discussion:** LQFM018 was cytotoxic to cells in a concentration manner, morphological changes with gain in cell volume (oncosis), plasma membrane rupture and subsequent loss of intracellular was observed. G2-M phase showed a significant increase in cell cycle analysis. Annexin analysis revealed a significantly ($p<0.0001$) increase of 33-fold in necrosis (A-/PI+). An increase in ΔΨm during the first 12 h was observed, however a significantly loss of ΔΨm (35.8%) was observed after 24 h treatment. The same pattern was observed with ROS. Cytochrome-c release and TNFR1 increased after LQFM018 treatment and caspase-3 and caspase-8 activities were slightly inhibited. Caspase-3, -8 and NF-kB mRNA levels were not significantly altered, but CYLD level increased. These data suggests that LQFM018 induced a regulated necrosis on the cells. Furthermore, LQFM018 affect CFU-GM and 3T3 cells viability at the low concentrations tested. This compound also showed low toxicity *in vivo (*category 5 of GHS, in agreement with OECD). **Conclusions:** LQFM018 has cytotoxic and immunosuppressive effects and the death process triggered by LQFM018 is regulated necrosis.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

MD 18- EFFECT OF WEATHERED ASSOCIATED WITH NANDROLONE TRAINING AND Lepidium meyenii IN RATS ON THE ACTIVITY ALANINE AMINO TRANSFERASE AND ASPARTATE AMINOTRANSFERASE

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Introduction: resistance training has become popular among academies, with greater demand for androgenic anabolic steroids (aas) in order to obtain faster results hypertrophy, muscle mass, strength and body remodeling. The use of anabolic usually occurs in combination with other substances, such as lepidium meyenii, which increase energy and vitality, can enhance the effects of the eaa. **Objective:** aim of the study was to evaluate the enzymatic activity of alanine amino transferase (alt) and aspartate aminotransferase (ast) of wistar rats submitted to the chronic resistance training associated with nandrolone and lepidium meyenii. **Materials and methods:** it used an experimental model with 60 adult rats, divided into groups: sedentary control (s), strength training (t), nandrolone decanoate (n), l. Meyenii (l), nandrolone decanoate and l. Meyenii (nl), strength training and nandrolone decanoate (tn) and strength training, nandrolone decanoate and l. Meyenii (tnl), lasting five weeks. The animals were subjected to a strength-training apparatus, through the squat, held three times a week. They received intramuscular injections of nandrolone decanoate, at a dose of 18mg / kg / week, and orally (gavage) maca supports at a dose of 450 mg/kg/week. Upon completion of the training the mice were euthanized, the truncal blood was collected to obtain serum and measurement of alt and ast enzymes by kinetic methods. The comparison of the parameters between groups were three-way anova and tukey post test. **Results anda discussion:** the obtained results demonstrated a significant decrease ($p < 0.05$) in alt activity in u groups (73.7 ± 2,7ui), n (53.0 ± 2,8ui), nl (54.0 ± 7 7 iu) vs. S (93.8 ± 7,8ui). The nl group had lower levels ($p < 0.05$) of alt in relation tn (84.5 ± 6,8ui). What about ast showed reduced rates ($p < 0.05$) in the nl (260.1 ± 23,5ui) versus s (368.2 ± 61,8ui) and tn (423.1 ± 28,5ui). **Conclusions:** the enzyme profile was observed that the performance of aminotransferases were changed, suggesting that the dose of the steroid and l. Meyenii and study time can be limiting factors to assess toxicity.

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MD 19- HEPATOTOXICITY EVALUATION OF THE NEOLIGNAN ANALOG 2-(4-NITROPHENOXY)-1PHENYLETHANONE

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Introduction: Neolignans are a group of secondary metabolites of plants that exhibit a great functional and structural diversity. In the group of oxineolignans, the neolignans 8-O-4' type are known to exhibit anti-inflammatory, antiplasmodial, antifungal, antioxidant and antitumor activities, and are promising new drug candidates. The Hepatic toxicity is the most usual adverse reaction to the use of drugs and is a major cause of death from drug toxicity. The morphometric analysis of the liver may show significant alterations, which can be noted from the analysis of different structural parameters that show the hepatotoxic effect from early changes in hepatic structure. **Aim:** The aim of this study was to evaluate the hepatotoxic effect of neolignan analog 2-(4-nitrophenoxy)-1phenylethanone (4NF) in mice. **Material and Methods:** The 4NF molecule was obtained by synthesis and characterized by nuclear magnetic resonance and infrared. Doses of 50, 75 and 100mg.kg^{-1} of neolignan analogue were administered 24 and 48 hours before the euthanasia. To evaluate the hepatosomatic Index (I.H.) the weight of the mice was obtained and then the animals were dissected and the liver were removed and their weight measured. The livers were fixed in paraformaldehyde and embedded in paraplast®. The 4µm slices were stained with hematoxylin and eosin and analyzed as the area of the hepatocyte nuclei, cytoplasm area, the number of Kupffer cells and other histopathological criteria. **Results and Discussion:** In the evaluation of I.H. the results showed a significant difference at a dose of 75mg.kg^{-1} for the two exposure times ($p<0.05$). In the histopathological analysis was observed a significant increase in the cytoplasmic area in the treatment of 75mg.kg^{-1}, and of nuclei area in 100mg.kg^{-1} and 75mg.kg^{-1} doses. Was observed an increased at number of Kupffer cells in the treatments with 100mg.kg^{-1} ($p<0.05$). It was observed steatosis in the hepatocytes of mice at all doses tested at 24 hours exposure, and at the dose of 75mg.kg^{-1} in 48 hours. The increase in the cytoplasm and the nucleus area can be considered a response to a stressor. It indicates an activation metabolic of hepatocytes in adverse conditions. Steatosis is a process that can be initiate by several factors leading to a deficiency of lipid metabolism and an inadequate activity developed by the liver. Several studies have linked the increased of Kupffer cells with pathogenicity of liver diseases. According to the literature there is a communication between Kupffer cells and hepatocytes to coordinate the storage of fat in the liver. **Conclusion:** This compound has moderate hepatotoxicity in the tested doses, causing steatosis. More studies are needed to better understand the mechanism on how the substance interacts in the body.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

MD 20- IDENTIFYING CASES OF DRUG INTOXICATION IN CHILDREN UNDER 5 YEARS

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Introduction: Intoxication can be understood as a clinical or biochemical event triggered by a chemical or intoxicating agent which is capable of promoting a reaction that causes damage in a living organism. The **objective** of this research was to identify which indexes observed in Brazil, Northeast, especially the state of Rio Grande do Norte (RN), related to drug intoxication in children between 0 and 4 years old. **Materials and Methods**: The study is quantitative approach based on data from the base of the National System of Toxic-Pharmacological Information (SINITOX), with analysis of data from descriptive and further development of absolute and relative frequency tables statistics. **Results and Discussion:** Cases of drug intoxication in children 0-4 years in the period 1999-2009 in Brazil obtained a total of 90,698 cases which proves the high incidence and prevalence in this age group. The drugs are the main cause of intoxicating agents in the above age range, followed by disinfectants, since age is characterized in the phase of curiosity, what makes the children to take it to the mouth. **Conclusions**: On the research, which proves this injury in high levels at ages 0-4 years, it is important that the services and institutions consider the greatest risk for intoxication in this age group, favoring development of prevention and health promotion actions, especially with families aimed at changing behavior.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

CLINICAL TOXICOLOGY

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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CL 01- INDIVIDUALIZED VANCOMYCIN DOSES FOR NEWBORN INFECTED PATIENTS

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Introduction: Vancomycin is effective against gram-positive bacteria and the first-line antibiotic for treatment of proven coagulase-negative staphylococcal infections. **Objective**: The objective was to investigate vancomycin dose adjustment in newborn infected by evaluating trough drug concentrations and the pharmacokinetic correlation. **Materials and Methods**: Study subjects included 02 patients with 75 and 89 days of born and normal renal function. Trough serum concentrations were measured prior administration of the 9th dose. Trough concentrations of 10 – 20 mg.L^{-1} were considered therapeutic. The patients were investigated prospectively. Plasma monitoring and PK assessments were performed by blood sample collections (3 sets for each patient) after steady state achievement (after four doses had been administered) and the vancomycin dose adjustment was made by USC Pack PC Collection software. Only 0.2 mL of each plasma sample was required for our plasma measurements by dry chemical. **Results and Discussion**: Trough values less than 10 mg.mL^{-1} was obtained in one set of one patient, as a consequence of increased plasma *clearance* and the apparent volume of distribution. The daily dose was subsequently increased from 15 to 17.4 ± 1.1 mg.Kg^{-1} during 15 days. Following the logistic regression analysis of treatment, new vancomycin target concentrations were estimated and used in development of an alternative dosing strategy. Simulation of a new dosing regimen yielded the following recommendations: 17.4 mg.Kg^{-1} at 36-h intervals. **Conclusions**: To adjust the vancomycin dosage regimen in pediatric infected patients with normal renal function, an initial dose of approximately 15 mg/kg/day was recommended; however, this dosage regimen should be further evaluated in this population in terms of efficacy and toxicity as well as in terms of achieving pharmacodynamics goals.

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CL 02- CORRELATION BETWEEN PLASMA TAMOXIFEN CONCENTRATION AND TUMOR RESPONSE IN PATIENTS WITH BREAST CANCER

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Introduction: tamoxifen (Tx) is currently the endocrine therapeutic agent of choice for all stages of breast cancer and has also been approved for use as a chemopreventive agent in women at high risk for the disease. **Objective**: to determine a possible correlation between the tumor response of patients with breast cancer initially treated with Tx and the plasma concentration of this medication. **Materials and Methods**: we studied 27 elderly patients (age range: 62 to 82 years) with advanced breast carcinoma who were treated with oral 20 mg.day^{-1} Tx, the dose considered to be ideal, for 3 months. Responders were followed up for 19 months and non-responders for 21 months. We measured plasma Tx citrate levels in plasma in order to determine their possible correlation with objective remission of the disease. **Results and Discussion**: the correlation was found to be significant among responders (37%), whose median plasma Tx level was 187.40 ng.mL^{-1}, as compared to non-responders, whose median plasma Tx level was 99.52 ng.mL^{-1}. The frequency distribution of patients in both groups with concentration of Tx lower and upper 182,60 ng.mL^{-1} was significant by Fisher's test ($p<0,0011$). **Conclusions**: on the basis of the present results, we suggest that patients whose plasma Tx levels reach 182,60 ng.mL-1 after 3 months of treatment, with no tumor response, should stop treatment because they will probably not benefit from it.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

CL 03- NEW REFERENCE VALUES FOR SELENIUM: FRONT RESPONSE TO NEW NEEDS

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Introduction: Selenium (Se) is a trace element found in nuts and vegetables has variable intake, depending on the availability of Se in soil. A deficiency can cause cancer, degenerative diseases, immune deficiency, arthritis and heart disease. Skin protective agent against UV rays. Absorbed in the intestine, transported in plasma by selenoproteins and excreted by the kidneys. Intoxication causes metallic taste, nails and brittle hair, respiratory irritation and increased AST / ALT. Sharply can cause seizures. In children excess selenium may slow growth. No occurrence of chronic poisoning in indigenous populations, regions whose soil is rich in selenium, and occupational exposures (semiconductor and cosmetics industry). In these cases absorption is respiratory or skin. The determination of selenium in serum is helpful in cases of intoxication or impairment. **Objectives:** propose a revision of the reference value (RV) for selenium dosages in serum. **Materials and Methods:** We analyzed results from the database of individuals who carried out this dosage in Fleury from January 2014 to May 2015. Selenium levels were determined by atomic absorption spectrophotometer, AAnalyst 800, Perkin Elmer, with graphite furnace and Zeeman effect. N = 17,198 samples and 12,350 women and 4,838 men and 940 children under 18 years. The results were processed by the EP *Evaluator* v.11 program and compared with the current VR - 46-143 µg/L -*Tietz Clinical Guide to Laboratory tests*. General Clinical Tests, 976. 2006 *Elsevier* 4ed. **Results:** This population had a selenium variation range of 25-1260 µg/L of which 15.6% were above the current reference value. Between men and women have not changed significantly, with 16.1% of men and 15.4% of women had their results changed. In less than 18 years, 98.3% had results within the expected range. The reference range proposal (with CI = 95%) is 63-197 µg/L. **Discussion and Conclusion:** It was observed difference between RV suggested by the EP Evaluator and current values. We observed an increase in demand for these dosages for other specialties that are not occupational medicine. So the focus moves from toxicology to nutritional aspects. Moreover, there are many patients, and nutritional supplementation might question excess of this limitation, or if monitoring of selenium is made appropriately. We note large number of patients with values above the reference mainly adults. Contrary to this the population aged under 18 showed fewer changes. We conclude that the current reference range is not adequate and should be reviewed using a systematic sampling best.

CL 04- COCAINE POISONING IN A NEWBORN CONFIRMED BY TOXICOLOGICAL ANALYSIS IN FOUR BIOLOGICAL MATRICES: CASE REPORT

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Introduction: The prevalence of cocaine use, as well as its alkaline product (crack), has increased in Brazil during last decades, also affecting obstetric population. Complications of drug abuse are not restricted to pregnant woman, but also reach the fetus, because most of those substances cross the placental barriers. Toxicological analyses in neonate matrices including blood, meconium, urine and breast milk may be useful to recognize cocaine prenatal exposure. **Objectives:** Case report of cocaine poisoning in newborn, confirmed by toxicological analyses. **Materials and Methods:** Retrospective case report of pre-term neonate, male, 35 5/7 AGA, 2810g, he was readily intubated after birth due respiratory distress, also presenting muscle spasms, hypoglycemia, hepatomegaly, and other symptoms related to neonatal infection. His mother reported to be a crack user for 16 years, and had last used the drug just 2 hours before the delivery. In the first 5 days of life, neonate evaluated with convulsion and septic shock. Toxicological analyses were performed in blood, urine, meconium, and breast milk by using immunoassay and gas chromatography-mass spectrometry (GC-MS). **Results and Discussion:** It was observed the persistence of cocaine metabolites in neonate urine and blood during at least 5 days, even after interruption of placental exposure. From the 1st to 5th day postpartum, toxicological analyses in urine demonstrated to be positive to cocaine metabolites in high concentrations (>900ng/mL), and blood analyses indicated that cocaine and metabolites were still present in his circulation for a relatively long period (benzoilecgonine 30ng/mL on 5th day). This finding corroborated with the fact that newborns have immature biotransformation system, especially with respect to cholinesterases responsible for the primary cocaine biotransformation. Detection of anhydroecgonine methyl ester in the newborn blood confirmed specific exposure to crack. It was also demonstrated his exposure during pregnancy by meconium analyses, that showed significant amount of cocaine metabolites (>10ng/g). The finding of cocaine traces in breast milk collected on the 3rd day proves this drug crossed mammary tissues, indicating it could be a potential secondary way of exposure. However, in this case the neonate was not breastfed by his mother, suggesting cocaine prenatal exposure was the single cause of poisoning. The newborn presented clinical improvement during the following days, and on 23rd day was discharged from intensive care. **Conclusions**: This case suggests the importance of toxicological analysis as tool for drug abuse diagnostic in the obstetric health system, providing adequate intervention to prevent, or mitigate neonatal complications.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

CL 05- ANALYSIS OF DATA ON RATIONAL USE OF ANTIDOTES FROM POINSON CONTROL CENTER IN BRAZIL, FOR USE IN PUBLIC HEALTH ACTIVITES

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INTRODUCTION: Currently, the poison cases register growth each year. This fact can be observed because have a lot of chemical substances exposure of the body and notification improvement of poison cases from Poison Control Center. The National Poisoning Information System register 99.016 poisons cases in Brazil, 2012, which 26.987 for medications, 20.793 for venomous animals, 4.657 for agricultural pesticides and 2.291 for rodenticides. This scenario showed above the rational use of antidotes becomes an important tool for Clinical Toxicology. **OBJECTIVE:** The aim of this work evaluates the dates of Poison Control Center in 2014 about therapeutical procedures as regards the rational use of antidotes before and after admission hospital care. **METHODOLOGY:** The study analyzed 1.037 records of telephone / personal assistance from Poison Control Center Antônio Pedro University Hospital, Niterói, Rio de Janeiro, Brazil between January to December 2014. The poisons studied were venomous animals, acetaminophen, benzodiazepines, organophosphates and coumarin of which 140 records were selected for a study. Moreover, the epidemiological dates were recorded. The program SPSS Statistics® Version 20 was applied. **RESULTS AND DISCUSSION:** The results revealed that the men were the most poisoned (53.6%). In regard age group, the children between 1-4 year showed 20.0% of poisons followed by the 20-29 year (16.4%) and 10-14 year (13.6%). The other important epidemiological parameter evaluated was intentionality of intoxication that unintentional was more prevalent (72.9%) with environmental mean origin (47 cases). The analyzed of selected poisons agents the principal class was venomous animals (39.2%), benzodiazepines (25.8%), organophosphates/coumarin (27.8%) and acetaminophen (7.2%). The use of antidotes was necessary for 45% of the poisons, which 21.4% of serotherapy, 13.5% atropine, 7.2% N-acetylcysteine, 1.4% of flumazenil and 1.4% k vitamins. The people that has antidote indication 20% does not follow the clinic. **CONCLUSION:** The current study allowed evaluated the rational use of antidotes from Poison Control Center even as mean intoxication causes and epidemiological profile of the population. The collect information object and monitoring of cases showed efficient for scenario building intoxication. Therefore, improvement politic public health for the rational use of antidotes on emergency care units.

CL 06- NTOXICATIONS BY CARBAMETE REGISTRED AT CIT-BELEM

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Introduction: The pesticide carbamate-based, popularly known as "chumbinho" is improperly used as rodenticide by great part of the Brazilian population, and is easily acquired by anyone, thus contributing to increasing rates of poisoning. The oral exposure to carbamate is responsible for inhibiting acetylcholinesterase, causing the victim to present cholinergic syndrome with demonstrations of muscarinic, nicotinic effects and acting on the central nervous system. **Objective:** Characterize the poisoning by carbamate, notified to the Toxicological Information Center of Belem (CIT- Belem). **Materials and Methods:** Descriptive study of cases of carbamate poisoning registered in the database of the CIT-Belem, located at the University Hospital João de Barros Barreto, in Belem of Para, Brazil, in the period from 2011 to 2014 and analyzed in the programs Epiinfo 6:04 and Excel 2010. **Results and Discussions**: In the period were reported 128 cases of carbamate poisoning, of which 57.04% were female, 39.06% were male and 3.9% was not informed the gender of the victim. Notifications assessed the highest percentage was from accidental origin with 49.21%, followed by suicide attempts with 43.75%. Of the 73 cases occurring in women, 52.05% were suicide attempts, where the young (11-22 years) represented 30.13% of the total. Compared to men, there were 50 cases, and the suicide attempt was present in 36% of the notifications; youth (11-22 years) accounted for 14% of this amount. The accidental poisoning source totaled 63 cases, of these, 36.32% were in children aged 0-10 years. Patients showed muscarinic and nicotinic symptoms as vomiting, diarrhea, drooling, dyspnea, numbness, tremors, cyanosis, tachycardia or bradycardia, bronchial hypersecretion; having 2.34% of confirmed deaths, whose victims were met with more than 12 hours of exposure. The cure rate was 57.03%, and in 35.15% the cure was not confirmed. **Conclusion:** Statistics have shown that more effective control measures to combat this sale must be taken, because accidents happen frequently, and there are high suicide attempts with the product. Poisoning by this substance causes symptoms of complicated treatment with cholinergic and systemic manifestations, with great probability of death. The study found that women were more susceptible to suicide attempts, and the large number of youth and adolescents, present in this statistic, is alarming.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

CL 07- POISON IN HOUSEHOLD CLEANING NOTIFIED IN CHILDREN IN TOXICOLOGICAL INFORMATION CENTRE OF BELEM

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Introduction: Household cleaning or sanitizing are substances or preparations used for cleaning or disinfecting collective or public environments. Examples of sanitizing detergents, bleaches, disinfectants, soaps, glass cleaners, insecticides, among others. These products, which by their composition can cause injury to the health of people presenting varying degrees of toxicity. **Objective:** To describe the profile of childhood poisonings recorded in household cleaning Toxicological Information Centre of Belém (Belém-CIT). **Materials and Methods:** A descriptive study of poisoning by household cleaning, considering the age group 0-10 years in the period from 2011 to 2014, whose information was obtained from the database of the Belém-CIT, the EPI-INFO program version 6.04 and analyzed in Excel. **Results and Discussion:** During the study period were 243 reported cases of toxic exposures in children, resulting in approximately 64.45% of all occurrences of poisoning by household cleaning. In the study of exposure pathways, proves the predominance of mouth with 95.06%, and the most common clinical manifestations of this type of exposure were nausea, vomiting, sore throat, abdominal pain, oral mucosal irritability, and dyspnoea. Sodium hypochlorite is the most frequent toxic agent in poisoning of about 49.38%. Regarding the condition, about 98.76% were single accidents, and only one case was a management error. There was a high rate of accidents in the age group 1-3 years, approximately 73.25%. The cure rate was approximately 68.31%, and healing unconfirmed 29.62%. There were no deaths. **Conclusion:** Household cleaning poisoning is very common in the age group of the study, and the mouth and gastrointestinal symptoms and the most frequent being the sodium hypochlorite as incriminated. This highlights the need for more effective preventive and educational measures, directed to the family aiming to inform and make them aware of the risks of exposure to these products, storage and proper use of household cleaning products and measures to be taken in the event of poisoning by these products.

CL 08- COMPLICATION IN CROTALIC ACCIDENT BY THE USE OF ANTI-ELAPIDIC SERUM: A CASE REPORT

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Introduction: In 2014, there were 455 reported cases of accidents with snakes in Ceará, Brazil. Snakes of *Crotalus* genera were responsible for 51 of these cases with 17 classified as moderate or severe. Its venom constitutes a toxic-enzymatic complex with neurotoxic, myotoxic and nephrotoxic actions. **Case report:** F.E.C.C 28 years old, male farmer dweller of Aratuba-Ceará, arrived at the hospital in Fortaleza-Ceará, five hours after crotalic accident presenting local erythema, bipalpebral ptosis, myalgia, asthenia, decreased visual acuity, dysphagia and vomiting episodes but normal urine color and blood clotting time. Ten ampoules (100 mL) of anti-Elapidic serum (AES) was administered via intravenous to the patient who presented an allergic reaction leading to general clinical complication. The patient presented blood clotting time alteration and oliguria with darkened urine needing hemodialysis. Twenty ampoules (200 mL) of anti-Crotalic serum (ACS) were administered to the patient and then he began to show improvement, but afterwards he developed hard controlling arterial hypertension and acute renal failure (ARF). Renal biopsy revealed acute tubular necrosis. The results of biochemical and hematological tests are following: leukocytes (22.890/mm3), TAP (13.5 s), TPTA (23.5 s), urea (96 mg/dL), creatinine (2.7 mg/dL), K^+ (7.42 mEq/L), PCR (136 mg/dL), CPK (>40.000 U/L), PA (184x89 mmHg). **Results and Discussion:** High CPK values result from injury of skeletal muscle fibers (rhabdomyolysis) caused by myotoxic action of venom with consequent release of enzymes and myoglobinuria, as evidenced by the appearance of dark red color urine. The presence of hyperkalemia, high levels of creatinine and urea associated with oliguria, indicate the onset of ARF, also evidenced by the development of hard controlling arterial hypertension. The ARF with tubular necrosis is the main complication of crotalic accident due to the direct action of venom on the kidneys and prerenal factors (renal hypo perfusion), as well as the toxic action of myoglobin on the renal tubules. The allergic reaction to anti-elapidic serum with possible deposition of immune complex in the glomeruli may have contributed to ARF. The similarity between enzymatic activity of crotalic and elapidic venom contributed to the erroneous administration of AES instead of ACS. **Conclusion:** Knowing the correct therapeutic approaches and the differences between clinical manifestations of crotalic and elapidic accident has a pivotal role in avoiding complications such as ARF or even death. Myoglobinuria and oliguria may not be the present even in severe cases of crotalic accidents. The dosage of CPK can be useful in differentiating these accidents, since it rises within 24 hours in crotalic accidents.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

CL 09- IMPLICATIONS ON ABUSIVE USE OF DIETARY SUPPLEMENTS

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Introduction: The use of dietary supplements without medical supervision has become more frequent among practitioners of physical activities. The death of a young woman in Fortaleza-Ceará brought up the discussion on this subject. She arrived at the hospital already unconscious and while receiving care, suffered two consecutive heart attacks. She was in a coma until her cerebral death has been confirmed. As she did not present any chronic pathology, the family suspected that this sudden illness has been caused by the use of several substances in intention to lose weight and increase her performance during physical exercises. **Objetcive:** To provide relevant information on the case in order to alert the public about the risks of using dietary supplements without medical supervision. **Methodology:** We conducted an active search of news on media about the case and a review of scientific literature on the subject. **Results and Discussion:** The patient used Lipo-6 Black Ultraconcentrate®, Franol® (Ephedrine + Theophylline) and an injectable substance not identified. The thermogenic Lipo-6 Black Ultraconcentrate® has caffeine and synephrine on its formulation. Caffeine, a common component in thermogenics for promoting lipolysis, has a stimulating action on the central nervous system leading to increased heart rate, blood pressure and body temperature. Synephrine has been used as a slimming based on its supposed thermogenic action caused by the stimulation of specific receptors mainly found in the liver and adipocytes leading to accelerated fat degradation and elevated caloric consumption. Previous studies have reported cardiovascular problems and cerebral ischemia as toxic effects related to continuous use of synephrine. In addition, toxic effects such as cardiac arrhythmias, tachycardia, hypertension, heart attack and stroke, due to association of synephrine and plant extracts containing caffeine, were reported in the literature even in patients without history of cardiovascular disease. The Franol®, drug indicated for the treatment of bronchial asthma and emphysema, became misused by people looking for a quick weight loss. It presents tachycardia, arrhythmia and hypertension as risks associated with its misuse. The combination of ephedrine and caffeine has been linked to adverse effects similar to those produced by the combination of synephrine and caffeine. **Conclusion:** Cardiovascular impairment is the main toxic effect of substances used by the patient, confirming the clinical picture presented by her. The medical report indicated that there was use of anabolic steroids, a fact that contributes to exacerbation of cardiovascular compromise.

CL 10- CHARACTERISATION OF TRAMADOL EXPOSURE REPORTED TO THE POISON INFORMATION CENTER, PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

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Introduction: Tramadol is a narcotic analgesic with a narrow therapeutic window. It is widely prescribed in adults for the treatment of acute and chronic pain, when NSAIDs and acetaminophen fail. In Chile, the Poison Information Center of the Pontificia Universidad Católica de Chile (CITUC) records around thirty thousand inquiries annually. **Objective**: The aim of this work was to characterize cases registered between 2006 and 2013 by CITUC whose etiologic agent is tramadol. **Materials and Methods**: An observational, descriptive and retrospective study was conducted. Data was collected between 2006 and 2013 using the DMS INTOX software. **Results and Discussion**: 1174 cases were received in the studied period, showing an upward trend starting with 66 cases reported in 2006 to 236 cases in 2013. In relation to the interlocutor, 70% were health professionals and 20% family members. Regarding the time between exposure and inquiry, 70% of cases were reported within the first 6 hours after intake, with 33% of the calls in the first hour after exposure. From the reported cases, 62% corresponded to women and 37% to men. Pediatric patients account for 30% of cases, scholars to 3%, teenagers to 7%, adults 50% and elderly at 8%. In pediatric population, 57% of these cases were accidental exposures while a 41% were misprescriptions. In adolescents 80% of inquiries were suicide attempts. In adult population, 44% of cases were suicide attempts, 18% adverse drug reactions, 16% misprescriptions and 13% were inquiries by self-medication. In the elderly, 33% of the consultations were due to therapeutic errors, 23% by adverse reactions, 17% by misuse and 11% for suicides. Around 0.1% of total cases were related to inquiries from abuse. Accidental tramadol ingestion by children and erroneous administration after confusing it with another drug are common situations that cause poisoning in children, especially in newborns, who can develop coma, seizures, respiratory depression and bradycardia at normal dosage in adults. In adults, a high amount of queries were due to self-medication or failure to medical indications, which may result in adverse reactions or poisonings. Unlike suicide, all causes of accidental exposures and poisonings are preventable with proper education. Tramadol users must be aware about potential risk of drug overdose and also accidental ingestion or erroneous administration in children through a proper storage and not to confuse it with a pediatric drops formulation. **Conclusions**: Tramadol is a widely used drug in clinical practice and the poisoning cases are increasing throughout the reported years. A high proportion of cases, both adults and children are due to errors in the administration of drugs and misuse, which are preventable through patient education.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 11- OCCUPATIONAL EXPOSURE TO PESTICIDES: A REPORT FROM THE POISON INFORMATION CENTER, PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE (CITUC).

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Introduction: Pesticide poisoning is included among occupational diseases and as a public health problem. According to national data, occupational cases account for 50% of total cases of pesticide poisoning. Occupational exposures occur mainly in field workers. In Chile, information available on this subject is scarce. To decrease occupational pesticide exposure is essential to develop safer work environments and better policies preventing permanent damage to workers. **Objective:** The aim of this work was to characterize occupational poisoning due to pesticide use from cases received by our poison information center between 2006 and 2013. **Materials and Methods**: A descriptive, observational retrospective study was conducted. Variables analyzed were agent family, route of exposure and sex. Analysis was carried out through frequency distribution and statistical association was assessed by calculating odds ratios with 95% confidence. Data were analyzed using SPSS 21.0 statistical software. **Results and Discussion:** CITUC received 1,718 calls of occupational exposures involving pesticides within the study period, equivalent to 30% of all cases of occupational poisonings (pesticide-related and non-pesticide related). By gender, male workers represented 79% of the pesticide occupational cases. Ninety percent of the cases involved exposure to a single agent with the main class being organophosphates (33%). This finding agrees with local studies and data from developing countries where organophosphates are widely used. An association between route of exposure and gender was observed where males showed a doubled risk of exposure to a pesticide through dermal route (OR 2.0; 95% CI: 1.28 to 3.13; $p < 0.05$) when compared to females and other routes of exposure. This higher prevalence of skin exposure could be attributed to handling, mixing and application of pesticides. **Conclusions:** From all pesticide occupational cases, organophosphates were the most prevalent. Exposures by dermal route in men is a preventable risk factor for handling pesticides. Due to intrinsic toxicity of organophosphates, importance of hazard communication and use of personal protective equipment are key elements to prevent pesticide poisoning.

CL 12- EVALUATION OF THERAPEUTICAL PROFILE OF SYSTEMIC LUPUS ERYTHEMATOSUS PACIENTS FROM ARAÚJO LIMA AMBULATORY

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INTRODUCTION: Systemic Lupus Erythematosus (SLE) is an inflammatory disease, chronic and autoimmune. Therapy consists mainly in inflammatory symptoms control and immunosuppressant for auto-antibody containment. Patients can have a variety of drugs in a single prescription, resulting in polypharmacy. **OBJECTIVE**: Evaluate the drug usage among SLE patients serviced in the Rheumatology Service in Araújo Lima Ambulatory, in the Getúlio Vargas University Hospital of the Federal University of Amazonas. **METHODS**: Prospective, with descriptive character, observational study. The population consists of SLE patients regularly serviced in the Rheumatology Service in the Araújo Lima Ambulatory. Patients over 18 years old, meeting four or more criteria from the American College of Rheumatology (ACR), at least one appointment and using at least one specific drug for SLE treatment were included in this study. Patients with mental illness, psychiatric disorder or cognitive dysfunction were excluded from this study. Interviews were realized with the patients, inviting their participation in this study and presenting the Consent formulary (TCLE) if accepted. After signing of the TCLE, patients answered a formulary about socioeconomic aspects, health, drug usage and psychoactive substances. It was observed the drugs in use, their adverse reactions and drug interactions. **RESULTS AND DISCUSSION**: Eight cases of verified SLE patients were analyzed, women with an average of 41.5 years old, according to literature the average age varies between 33.2 and 41.5 years. The average of drugs used by patients was 7, being that all of them make use of prednisone, the second most used is chloroquine, as seen in other works, in which the most used drugs are glucocorticoids and posterior antimalarial drugs. About 62.5 %(5) reported quitting the treatment due to feeling ill. From the total 87.5 %(7) reported adverse reactions to the drugs, in which stomach ache and blurred vision were the most related ones. In relation the drug interactions 17 were classified as moderate, 2 as secondary and 2 as important reactions, being that the most observed interaction was between prednisone and hydrochlorothiazide. **CONCLUSION**: The identification and evaluation of the therapeutical profile of patients contributes with an important knowledge feed that allows for the diminishing of problems during treatment and prevent additional assistance costs, providing than a better quality of life to the patients.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
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CL 13- AVAILABILITY OF ACETAMINOPHEN BLOOD LEVELS IN THE PUBLIC HEALTH NETWORK IN CHILE

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Introduction: Acetaminophen (ACM) is a non-opioid analgesic widely used in Chile. In the UK and the USA, ACM poisoning is the most common cause of liver failure. In Chile, approximately 1000 cases of ACM overdose are reported every year, 25% of them associated to toxic doses. ACM poisoning management is often composed of decontamination and treatment with n-acetylcysteine (NAC) following a 72-h schedule. Recently, a shortened protocol has been recommended. While keeping its efficiency, it may reduce NAC adverse reactions and patient in-hospital stay. This novel protocol requires continuous monitoring of ACM blood levels (ACMbl). **Objective:** The objective of this work was to assess capabilities to determine ACMbl in the public health network for the application of the novel protocol. **Materials and Methods**: During January 2015 a survey was conducted to all high-complexity hospitals in the public network to assess their current capacity to measure ACMbl. Also, geographical distribution of ACM overdoses was extracted from reported cases (2006-2013) to the Centro de Información Toxicológica Pontificia Universidad Católica de Chile. **Results and Discussion:** From the survey only 3 out of 63 hospitals reported current capabilities to measure ACMbl which where localised in the central-south area of the country. This area accounts for 19,6% of the reported cases. In Chile, ACM poisoning occurs both from accidental ingestion and suicidal attempts throughout the country. Results from the survey showed that a limited number of hospitals have current capacities to measure ACMbl, and geographically restricted. Having capabilities to determine ACMbl is useful for both diagnosis and treatment of ACM overdose, and therefore a helpful tool for physicians. In the emergency room, ACMbl provides a real view of patient's status in those who decontamination measures have been performed. Finally, the use of ACMbl for the shortened protocol helps in optimising therapy and to potentially reduce adverse effects due to NAC therapy. Als,o it may help reducing in-hospital stay in ACM overdoses. **Conclusions**: Currently, 3 out of 63 high-complexity hospitals report capabilities to measure ACMbl. These hospitals may cover less than 20% of the overdose cases, and therefore this novel protocol may not be applied in large extent. Clinical utility of ACMbl is warranted but further cost-benefit analysis is required to provide full evidence for its wider implementation.

CL 14- THE EFFECTS OF REPEATED EPISODES OF BINGE DRINKING ON LIPID PEROXIDATION AND LIVER TISSUE DURING ADOLESCENCE TO ADULTHOOD

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Introduction: Alcohol abuse is a serious public health problem because it induces considerable deleterious effects in various tissues, especially in the liver. Binge drinking is the high consumption of this drug in a short period, commonly practiced by teenagers, and which grows among young women. This category of consumption tends to continue into adulthood, occurring in multiple episodes. **Objective:** Investigate the effects of binge drinking during adolescence until adulthood and after the late cessation, in female rats. **Material and methods:** Eighty adolescent female Wistar rats (35-days-old) were assigned into eight groups (n = 10/group): control groups (C1, C4, C8 and CW) and ethanol groups (E1, E4, E8 and EW). Animals received distilled water or ethanol (3 g/kg/day) by gavage for 3 consecutive days/week for 1, 4, and 8 binges episodes and 14 days without any administration. 24 hours after treatment period, rats were sacrificed. Blood samples and liver was collected for measurement of serum activity of aspartate (AST), alanine aminotransferase (ALT), and malondialdehyde levels (MDA). Other animals (n=5/group) were perfused and liver were removed for histopathological analysis. Ethical committee (BIO 196-14) approved all procedures. Statistical analysis were performed using Student's t-test, adopted $p<0.05$, and data presented as mean ± S.E.M. (n= 5-10 rats/group). Histopathological evaluation was performed qualitatively. **Results and discussion:** Body weight showed no deficits after the first binge drinking episode (E1) [72.42 ± 1.294 g, p=0.7867] and the last (E8) [158.6 ± 3.539 g, p=0.2709], indicating that this drinking pattern does not cause malnutrition. ALT serum activity was decreased in E4 [29.33 ± 3.208 U/L, p=0.0156] suggesting ALT synthesis inhibition. Both serum transaminases activity were higher in EW [ALT: 33.00 ± 6.351 U/L, p=0.0141; AST: 22.50 ± 2.276 U/L, p=0.2892], showing liver damage. Liver MDA levels decreased in E1 [9.205 ± 1.018 nmol/mg of protein, p= 0.0151] suggesting inhibition of lipid peroxidation, but increased in EW [5.072 ± 0.2583 nmol/mg of protein, p= 0.0336]. We suggest that such effects is resulted of an effective antioxidant action of estrogen elevation caused by alcohol consumption that are related to its pro-oxidant role. Ethanol groups showed microvesicular and macrovesicular steatosis, fibrosis or sinusoidal dilatation in the liver in all periods. **Conclusion:** The exposure to a single episode of binge drinking in the adolescent female rats alters biochemical parameters and liver tissue. Repeated episodes from adolescence to adult life demonstrate tendency of damaging in the recovery process illustrated by fibrosis. Ethanol withdrawal after repeated exposure promotes metabolic decompensation related to increased lipid peroxidation and steatosis.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 15- EVALUATION OF DEPRESSIVE SYMPTOMS IN FEMALE RATS AFTER EXPOSURE TO ETHANOL BINGE DRINKING FROM ADOLESCENCE TO ADULT LIFE

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Introduction: Ethanol (EtOH) is a worldwide psychoactive substance which the abuse is a serious public health problem. It induces considerable deleterious effects in various tissues, including the Central Nervous System (CNS). Binge drinking is a pattern of drinking that occurs a high consumption of this drug in a short period that can lead to intoxication, predominant in adolescents and young adults. The alcoholic beverage consumption has been increasing especially on female gender. Some studies showed the relationship between binge drinking and depression symptoms in humans, with alcoholism comorbid and depression. **Objective:** Evaluate behavioral changes related to depressive symptoms in rats after ethanol exposure in a binge pattern. **Material and method:** We used 20 Wistar female rats, divided in two groups: control and EtOH, in which received by oral gavage EtOH 3g/kg/day during three consecutive days, once a week during 35 days of treatment. Twenty-four hours after the treatment period the animals were submitted to behavior tests: Splash test (ST) and forced swimming test (FST). Statistical analysis was performed by Student-t test. **Results and discussion:** The evaluation of anhedonic behavior-like was made by ST, in which showed a reduction on the latency time ($p<0.05$; $t=2.996$) and in the grooming time ($p<0.05$; $t=3.355$) on the EtOH group compared to Control. This decreased on grooming time indicates a loss of self-care and motivacional behavior, sugestive of anhedonic-like behavior. After this, it was evaluated the depressive behavior-like accessed by FST. In this behavioral test the EtOH group did not show difference compared to Control group neither on immobility time or mobility time. **Conclusion:** Our results demonstrated that there is no occurrence of depressive-like behaviour after this alcohol paradigm model. However the results show a tendency to depressive disorders with symptoms such as anhedonia due to this early exposure to ethanol binge pattern.

CL 16- ACCIDENTAL INGESTION OF CAUSTIC INDUSTRIAL USE. TWO CASE REPORT

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Introduction: the accidental ingestion of caustic in pediatrics remains a serious problem. Most cases are because of transfer thereof to beverage bottles. Currently the severity of symptoms is due to industrial products. Objective: to report two cases of ingestion of alkalis and acids for industrial use and its evolution. Emphasize preventive measures.

Clinical cases:

Case 1: male patient, 4 years old, entering the emergency service by accidental ingestion of an industrial alkaline degreaser, transfered it into a bottled of water. Formula: sodium hydroxide 35%, sodium metasilicate 5.1% and sodium gluconate 5.1%. He presented serosanguinous drooling, sialorrhea, mouth and gum edematous, blackish white lesions and bald tongue. Ugi endoscopy: injuries grade ii in esophagus, injuries grade iii in stomach. Treatment was with corticosteroids, antibiotics, analgesics and omeprazole. It evolved with stenosis was required dilatation and restenosis. Currently with feeding tube and stenting.

Case 2: male patient, 8 years old, drank a sip of engine cleaning acid, transfered into energy drink bottle. Formula: 10% hydrochloric acid and 5% phosphoric acid. It presented vomiting, difficulty breathing, tongue and mouth erosion, drooling, longitudinal erythematous lesions in bald tongue. Ugi endoscopy: lesions in the esophagus grado ii a and ii b in its entirety, ijuries grade i in stomach. Medicated with omeprazole, antibiotics and sucralfate. Videodeglucion, 15 days after: delayed gastric emptying. Is performed at 30 days second ugi endoscopy: mild erythema in esophagus, 18 cm. Punctate stenosis, tutor probe is placed for feeding. Dilation of stenosis.

Conclusion: implementing prevention and awareness campaigns regarding the transfer and use of industrial products at home, considering the severity of symptoms.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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CL 17- CHEST PAIN RELATED TO COCAINE USE: CASE SERIES

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Introduction: Cocaine is one of the most abused drugs in the world, and it is a frequent cause of visits to emergency rooms. The chest pain or discomfort are usual complains, and may indicate acute coronary lesion. However, those symptoms should be evaluated with caution, as transient changes in the electrocardiogram (ECG) due vasospasm are common, and can easily be misinterpreted. The acute Myocardial Infarction (MI) related to cocaine often described in the literature appears as an unusual complication of the poisoning. **Objectives:** Case series report with typical chest pain related to cocaine use. **Materials and Methods:** Retrospective report of six cases of cocaine poisoning. **Results and Discussion:** Patients evaluated included 4 male/2 female, the average age was 31y.o. (21 to 42y.o.); the time between last exposure and admission at emergency room was 5h and 30min (range of 1h 40min to 12h). Regarding the pain location, 5 patients reported chest pain only, and one reported chest pain radiating to the left arm and back. Other reported symptoms were tachycardia (4), sudoresis (4), vomiting (3), dyspnea (3), nausea (2), hypertension (1), and dysphagia (1). All patients had negative results of laboratory analyses for myocardial necrosis (CPK, CPK-MB and Troponin); the electrocardiograms (ECG) did not show ST-segment elevation or depression, or T wave inversion. Blood and urine samples analyzed by GC-MS showed the following results: in one blood sample was found detectable levels of Cocaine (COC) 1.2 ng/ml, all other substances searched in blood were negative, including Cocaethylene (CE), and Anhydroecgonine methyl ester (AEME). Urine samples showed results of COC 793.6 ng/mL (59.1 to 2182.0), CE 767.4 ng/mL (32.5 to 3455.4), and AEME 613.7 ng/mL (2.7 to 3347.2). In only one urine sample CE and AEME were not detected. Benzoylecgonine (BE) was investigated only in urine, and was found positive in all samples with the average result of 8155.3 ng/mL (977.8 to 16019.9). Patients were discharged less than 24h after admission asymptomatics. **Conclusions:** Patients evaluated with typical chest pain have not confirmed MI in this series, and showed favorable outcome of poisoning with symptomatic treatment, including diazepam. They looked for medical advice in approximately 5 hours after the last exposure, or later, which may have influenced the results found in blood. It was not possible to establish any relationship between blood concentration and the severity of clinical manifestations. Results in urine positive for CE and AEME indicated that the association of cocaine, crack and alcohol is possibly very common. Further prospective studies with larger samples are needed for better understanding of chest pain related to cocaine use.

CL 18- COMPARATIVE STUDY OF N-ACETYLCYSTEINE USE IN PARACETAMOL POISONING BETWEEN THE POISON CENTER OF SÃO PAULO, BRAZIL, AND THE POISON CENTER OF MONTEVIDEO, URUGUAY.

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Introduction: The Poison Control Center of São Paulo (CCI), and Poison Information Center of Uruguay (CIAT) are located in 24 hours general hospitals. Their cases records show that CCI had 5080 consultations during the first 8 months of this year (2015), while CIAT had 6992 consultations in the same period. **Objectives:** Compare the patient profile and results of different administration protocols of N-acetylcysteine (NAC) between the two Poison Centers (PC). **Materials and Methods:** Retrospective, observational, descriptive clinical study of paracetamol poisoning cases reported in Sao Paulo and Montevideo, from January to August, 2015. Only the cases where NAC was used in the treatment have been selected for this study, and the following variables were considered: gender, age, estimated doses ingested, serum paracetamol level evaluated according to Rumack-MatthewToxicicity Nomogram, latency of the query to the PC, circumstance of exposure, route of the antidote administration, and clinical severity (Poison Severity Score, WHO). **Results and Discussion:** In the period of the study, CCI answered 110 communications (2%), and CIAT 27 (0.4%) related to paracetamol. The number of cases that used NAC was 6 CCI and 10 CIAT. In both Centers prevailed females (70%), and young patients (19 y.o.). In 70% of the cases registered from CCI, and 90% of the cases from CIAT, the circumstance of poisoning was suicide attempt. Latency of the query to CCI was 10h (1h - 24h), and to CIAT 4h (20min - 9h). The estimated dose for the CCI cases ranged from 169 - 240mg/kg and for CIAT 152 - 700mg/kg. At the time of consultation, NAC was administered to 6 cases in CCI and 10 cases in CIAT, according to estimated doses ingested. CCI indicated intravenous (IV) NAC and CIAT indicated orally. One case with oral NAC treatment presented vomiting. No other adverse effects were reported in either set of cases. The final severity classification of poisoning registered in CCI was 1 severe case, and in CIAT non severe case was reported. All treatments exhibited good performance in both PC. **Conclusions:** Both oral and IV NAC treatment showed same efficacy, and no significant adverse effects were observed. There were no fatalities. These findings were similar to results found in the literature related to the profile of paracetamol poisoning, and therapeutic responses for oral and IV NAC treatments.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

CL 19- CHRONIC TRANS-PLACENTAL EXPOSURE TO ORGANOPHOSPHATE INSECTICIDE: A CASE REPORT

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Introduction: Pesticides are widely used in agriculture, livestock, Public Health campaigns and in the household. Recent research from the National Health Surveillance Agency - Program Review Residues in Food (PRRF/ PARA-ANVISA,2012) found that a third of the food consumed in the country were contaminated by pesticide residue with values above "acceptable" references. **Objective**: Study of a case report of trans-placental chronic exposure to organophosphate insecticide. **Method:** Consulting medical files from CEATOX and Botucatu Medical School Hospital**. Results:** In this study it was reported the following clinical case: M.S.S. 31 years old, woman, coming from Bofete-SP, with headache, dizziness, nausea, vomiting, muscle weakness and rash a few months ago. He worked from June 2008 to June 2010 as inspector of pests in orange plantation. It was established the nexus of chronic exposure to pesticides by qualitative toxicological analysis: June 2010 (positive organophosphate; organochlorine and carbamate undetected); November 2010 (not detected pesticides); November 2011 (positive organophosphate; organochlorine and carbamate undetected); August 2012 (undetected pesticides); and qualitative and quantitative analysis from February 2013 (Malathion organophosphate 64.8 ppb - 10.0 ppb detection limit); June 2014 (Malathion 33.3 ppb); February 2015 (Malathion 21.1 ppb - keeping dizziness and itching, with partial improvement of headache and total improvement of nausea and vomiting). Patient became pregnant in the second semester 2012, with child birth J.S.S.M. in August 2013 (Malathion 57.6 ppb in umbilical cord and placenta 74.0 ppb). The tests were repeted in the child in June 14 (Malathion 34.0 ppb) and February 2015 (Malathion <10.0 ppb). Children were exclusively breastfed up to 3 months old. **Discussion:** The positivity of patient exams, even after periods without organophosphate detection, is justified by the relationship between blood levels and stocks in adipose tissue, with redistribution between tissues. It is a case of chronic transplacental transmission of organophosphate (malathion), with mother´s blood levels similar to umbilical cord, with placental accumulation, and the same decay rate after ten to eighteen months of the child's birth. **Conclusion:** despite the wide use of pesticides, we found only three similar case reports in the literature, all related to acute exposure. It is a little known and underdiagnosed condition, with important repercussions for pregnant women and for the embryo / fetus, with the possibility of irreversible sequelae, such as congenital anatomical and functional changes and delayed neuro-psychomotor development.

CL 20- PROLONGED USE OF N-ACETYLCYSTEINE IN SEVERE PARACETAMOL POISONING ASSOCIATED WITH OTHER DRUGS: CASE REPORT

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Introduction: Paracetamol is widely used in both over the counter and on prescription pain relief drug. However, it has relatively narrow safety margin, and accidental or deliberate overdose may result in hepatic and renal toxicity. For repeated ingestions, the dose and time course of exposure required to produce hepatic toxicity are unknown. **Objectives:** Description of late therapy with N-acetylcysteine (NAC) indicated for hepatic dysfunction due to severe paracetamol poisoning associated with other drugs. **Materials and Methods:** R.E.F., male 32 y.o., in therapeutic use of tetracycline and dapsone for severe dermatological disease, suffering from depression, recent hospitalization due to Fournier Syndrome caused by secondary infection from anabolic steroids self-administration. He had used analgesic drugs excessively without prescription for several days, admitted at emergency room after repeated ingestions of unknown amount of paracetamol and morphine for the last 48 hours, presenting stupor, respiratory distress, intense oral and nasal bleeding. **Results and Discussion:** Initial laboratory analyses revealed hepatic and renal dysfunction (AST 7429U/L, ALT 5534U/L, Creatinine 2.8mg/dL, Urea 98mg/dL), altered coagulation (PT 54.5s, INR 4.54, aPTT 31.3s), and severe anemia (Hb 6.9g/L, Ht 21%). Toxicological analysis showed paracetamol serum levels of 5 µg/mL after more than 24 h from the last ingestion, diazepam 0.1 ug/mL, amitriptyline 0.3 ug/mL, morphine and codeine positive in urine. Late NAC therapy was initiated according to Prescott IV protocol, followed by continuous infusion of 150mg/kg/24h until normalization of hepatic function. He received hemoderivates and vitamin K to correct anemia and clotting disorder from 1st to 4th day of hospitalization (DH). Hemodialysis was performed in alternates days between 6th to 10th DH due renal failure; patient was under mechanical ventilation from admission up to 9th DH, when was extubated successfully. On 11th DH, therapy with NAC was discontinued; on 19th DH, patient was discharged with clinical improvement and the following laboratory results: PT 12.6s, INR 1.18, aPTT 29.1s, AST 45U/L, ALT 148U/L, Creatinine 1.7mg/dL, and Urea 27.7mg/dL. **Conclusions:** Therapy with NAC with the low paracetamol serum levels found in this case was important due the multiple and elevated doses taken. Hepatotoxicity and nephrotoxicity observed may have not been attributed exclusively to paracetamol, as the association with other drugs and the background of drugs and steroids abuse possibly worsened the hepatic injury. The late beginning and prolonged use of NAC may have contributed to improvement of liver and kidney function as described in literature, well evidenced by the final laboratory analyses.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 21- PROLONGED USE OF N-ACETYLCYSTEINE IN SEVERE PARACETAMOL POISONING ASSOCIATED WITH OTHER DRUGS: CASE REPORT

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Introduction: Paracetamol is widely used in both over the counter and on prescription pain relief drug. However, it has relatively narrow safety margin, and accidental or deliberate overdose may result in hepatic and renal toxicity. For repeated ingestions, the dose and time course of exposure required to produce hepatic toxicity are unknown. **Objectives:** Description of late therapy with N-acetylcysteine (NAC) indicated for hepatic dysfunction due to severe paracetamol poisoning associated with other drugs. **Materials and Methods:** R.E.F., male 32 y.o., in therapeutic use of tetracycline and dapsone for severe dermatological disease, suffering from depression, recent hospitalization due to Fournier Syndrome caused by secondary infection from anabolic steroids self-administration. He had used analgesic drugs excessively without prescription for several days, admitted at emergency room after repeated ingestions of unknown amount of paracetamol and morphine for the last 48 hours, presenting stupor, respiratory distress, intense oral and nasal bleeding. **Results and Discussion:** Initial laboratory analyses revealed hepatic and renal dysfunction (AST 7429U/L, ALT 5534U/L, Creatinine 2.8mg/dL, Urea 98mg/dL), altered coagulation (PT 54.5s, INR 4.54, aPTT 31.3s), and severe anemia (Hb 6.9g/L, Ht 21%). Toxicological analysis showed paracetamol serum levels of 5 µg/mL after more than 24 h from the last ingestion, diazepam 0.1 ug/mL, amitriptyline 0.3 ug/mL, morphine and codeine positive in urine. Late NAC therapy was initiated according to Prescott IV protocol, followed by continuous infusion of 150mg/kg/24h until normalization of hepatic function. He received hemoderivates and vitamin K to correct anemia and clotting disorder from 1st to 4th day of hospitalization (DH). Hemodialysis was performed in alternates days between 6th to 10th DH due renal failure; patient was under mechanical ventilation from admission up to 9th DH, when was extubated successfully. On 11th DH, therapy with NAC was discontinued; on 19th DH, patient was discharged with clinical improvement and the following laboratory results: PT 12.6s, INR 1.18, aPTT 29.1s, AST 45U/L, ALT 148U/L, Creatinine 1.7mg/dL, and Urea 27.7mg/dL. **Conclusions:** Therapy with NAC with the low paracetamol serum levels found in this case was important due the multiple and elevated doses taken. Hepatotoxicity and nephrotoxicity observed may have not been attributed exclusively to paracetamol, as the association with other drugs and the background of drugs and steroids abuse possibly worsened the hepatic injury. The late beginning and prolonged use of NAC may have contributed to improvement of liver and kidney function as described in literature, well evidenced by the final laboratory analyses.

CL 22- CASE REPORT OF RHABDOMYOLYSIS INDUCED BY CIPROFIBRATE

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Introduction: In March 2014, the Poison Information Center of Rio Grande do Sul (CIT/RS) met a case of rhabdomyolysis induced by therapeutic use of Lipless®. This product contains ciprofibrate, which has lipid-reducing properties with actions on plasma lipids; thus, it is indicated for the treatment of isolated severe hypertriglyceridemia or mixed hyperlipidemia, when statin is contraindicated or not tolerated. Among the adverse reactions mentioned in the package leaflet, there are some considerations about myalgia, myopathy, myositis and isolated cases of rhabdomyolysis. It is recommended that patients should be instructed to promptly inform their doctor about occurrence of myalgia, muscle discomfort or weakness. Muscle events seem to be dose-related and, therefore, the daily dose of 100mg should not be exceeded.[1] **Objective**: Make an alert to the importance of this adverse effect of ciprofibrate, since there is little information in literature about it - as evidenced after search in PubMed database (access on 12.03.2015) for the words "(ciprofibrate) AND rhabdomyolysis", when only 16 results were found. **Materials and Methods**: We reported a case of rhabdomyolysis induced by ciprofibrate attended by a CIT/RS call. **Results and Discussion**: Male patient, 63 years old, 90 kg, receiving ciprofibrate in therapeutic doses (100mg daily) for 20 days, arrived in an emergency unit presenting myalgia, paresthesia in fingers and loss of strength in the arms and legs. The medication was discontinued, but 4 days after the patient sought emergency care again complaining about the same symptoms. The emergency doctor asked for help to our Poison Center because the patient's creatinophosphokinase (CK) reached 1.807 U/L (normal range for man: 55-170 U/L).[2] He was advised to monitor CK and renal function and avoid to reintroduce ciprofibrate. In a review of 2009, 76 rhabdomyolysis cases related to fibrates were described, among them one produced by fenofibrate.[3] Rhabdomyolysis is a serious adverse event associated with the use of fibrates alone or in combination with drugs, so it is necessary to monitor patients who are using this kind of therapy and explaining for patients the risks of this treatment. **Conclusions**: Regarding this recognized adverse effect, there are case reports of rhabdomyolysis as a result of ciprofibrate use. Therefore, it is important to promote deeper studies on this subject. In addition, it is evident the importance of Poison Information Centers not only in aid of poisoning calls, but also in pharmacovigilance.

References:

1. LIPLESS® - ciprofibrato - Biolab Sanus Farmacêutica Ltda, 2013.
2. Register number 2014-04922 of Poison Information Center of Rio Grande do Sul, 2014.
3. Wu, J. *et al*. *Eur J Clin Pharmacol* **65**:1169–1174, 2009.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 23- CLINICAL AND LABORATORY CHARACTERISTICS OF PATIENTS INTOXICATED BY CHOLINESTERASE INHIBITORS ADMITTED TO A REFERENCE TOXICOLOGICAL ASSISTANCE CENTER IN NORTHEAST BRAZIL

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Introduction: Cholinesterase inhibitors (CI) intoxication is a very frequent poisoning in our region. It is observed in both accidental ingestion and suicide attempts. **Objective:** To describe the clinical and laboratory characteristics of CI intoxications. **Materials and Methods:** This is a cross-sectional study conducted with all patients victims of intoxication by CI admitted at the Toxicological Assistance Center at Instituto Dr. José Frota in the period from November 2013 to December 2014. Clinical and laboratory characteristics recorded at admission and during hospital stay (when hospitalization was required) were reviewed. Statistical analysis was performed with SPSS program v. 20, and p value < 0.05 was considered significant. **Results:** A total of 136 CI intoxicated patients were admitted in the study period, with mean age of 29.5±15 years, and 48.5% were females. In 119 cases (87.5%) the intoxication was intentional, characterizing suicide attempt. For the majority of patients (85.3%), hospitalization was necessary, with a mean time of hospital stay of 6.1±6.0 days. Clinical manifestations at admission were: neurologic symptoms (45.6%), myosis (44.1%) and gastrointestinal abnormalities (23.5%). Fever was observed in 27.2% of cases, and for 47% antibiotics were required. Mechanical ventilation was necessary for 52.2% of cases, and 11% used vasoactive drugs. Gastric lavage was performed in 59.5% of cases, activated charcoal in 66.2% and atropine in 63.2%. Regarding laboratory evaluation, the mean serum cholinesterase at admission was 281.5IU/l (minimum 58IU/l and maximum 13,000IU/l), and at the time of outcome it was 2,149IU/l (minimum 45IU/l and maximum 11,573IU/l). Leukocytosis > 12,000/mm³ was observed in 48.5% of cases; 22.9% presented acute kidney injury and 41.1% had hydroelectrolytic disturbances related to sodium and potassium. Regarding the outcome, 66.2% were discharged after normalization of cholinesterase levels, 8.1% had prolonged hospitalization due to different clinical complications, and 3.7% (n=5) had a fatal outcome. **Conclusion:** CI victims profile is characterized by young individuals, with no gender predominance. These patients, as a whole, have significant symptoms and require hospitalization and decontamination measures. Despite the high levels of clinical complications, including secondary infections, kidney injury and hydroelectrolytic disturbances, there is a low mortality. One important factor that calls out attention is the high rates of suicide attempts. Further social, psychologic and cultural studies are necessary to better understand factors that lead these people to suicide attempt.

CL 24- ACUTE KIDNEY INJURY AND OTHER COMPLICATIONS OF LOXOSCELISM – A CROSS-SECTIONAL STUDY IN NORTHEAST BRAZIL

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Introduction: Loxoscelism is described as the most severe form of spider bite in Brazil. There are two main forms: cutaneous, characterized by local pain, and cutaneous-visceral, which causes systemic signs of hemolysis, disseminated intravascular coagulation and acute kidney injury (AKI). **Objective:** To describe the occurrence of AKI and other complications among victims of spider bites. **Materials and Methods:** This is a cross-sectional study including all patients admitted to the Toxicological Assistance Center at Instituto Dr. José Frota, victims of spider bites in the period from January 2010 to June 2015. Clinical and laboratory characteristics recorded at admission and during hospital stay (when hospitalization was required) were reviewed. Statistical analysis was performed with SPSS program v. 20, and p value < 0.05 was considered significant. **Results:** A total of 42 cases were recorded in the study period. Patients' mean age was 32.7 years, and there was a predominance of females (64.3%). The majority of cases occurred in urban areas (81%), 38.1% in the city of Fortaleza. The most injured body sites were the lower limbs (40.4%). The characteristic necrotic skin lesion was observed in 95.2% of cases. Mean time between the accident and medical care was 125 hours. AKI was observed in 5 patients (11.9%), and they were classified as AKIN 1 (3 cases) and AKIN 3 (2 cases); 19% had coagulation abnormalities. Around 54% of patients presented secondary infectious complications, and 2.3% developed kidney injury requiring dialysis. Antibiotics were required for 61.9% of cases. Mean levels of serum urea and creatinine were, respectively, 38.5mg/dl and 1mg/dl, and mean values of prothrombin time and partial thromboplastin time activation were, respectively, 17s and 28s. **Conclusion:** Loxoscelism is more frequente in urban áreas in our region, causes necrotic cutaneous lesions in almost all cases and the main complications are coagulation abnormalities and kidney injury.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 25- STUDY OF BIOMARKER, GLUTATHION AND ITS CORRELATION WITH CHEMICAL SUBSTANCES

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INTRODUCTION: There are several scientific publications that link up the effects of biochemicals, xenobiotics or neurologic pathologies, to the levels of glutathione (GSH). In this study, executed between 2013 and 2015, we correlate the values under the normal parameters with the causes that motivated the specific toxicological analysis. The alterations of intracellular GSH are present during diverse pathologies and these are related to several apoptotic effects. This programmed cell death is associated to several diseases as cancer, neurodegenerative and autoimmune disorders. Thus initial studies suggest that GSH depletion was only a byproduct of oxidative stress, generated during cell death, recent studies suggest that GSH depletion and post-transitional changes of proteins though glutathionilation are critical regulators of apoptosis. **METHODS:** 64 cases from spontaneous patient were studied for toxicological effects of xenobiotics in biological matrix, and other healthy patients (HP) for insurance companies. **RESULTS:** The GSH blood levels were quantified, and the normal reference values (16,71 – 37,74 mg/dL) for this population were established. The age from the patients was from 2 to 77 years; 70% were women and 30%, men. The distribution was: 18,75% metals, 17,18% healthy, 10,94% acetaminophen (APAP), 10,94% cancer, 9,38% THC, 9,38% other drugs (OD), 6,25% fibromyalgia (FM), 4,69% dietary factors, 4,69% pesticides, 3,13% solvents, 3,13% tobacco, and 1,56% metahemoglobinemia (METHB). All patients signed informed consent and their results were given to them. In 3 cases of breast cancer, patients received, after the results, dietary supplements, and in occupational exposition to inorganic As and cardiovascular effects, were also supplemented, all of them with further controls that showed recovery up to normal values. From the relation between the agent and low levels of GSH, stand out: all cases of THC, levels under normal value; 57% of breast cancer patients; 50% of metals; for APAP, 42,58%; and in pesticides, 33%. With lower impact in GSH were OD (16,6% of them), and without impact in GSH, METHB, tobacco and FM. In HP, only 18% of them had low values of GSH, due to genetic and/or unknown dietary cause. **CONCLUSION:** In this study, the effects of THC on GSH are very important, considering that all the cases had alterations in the values of this biomarker, and the values were the lowest. The GSH depletion during xenobiotic-induced apoptosis, or other have been also reported for THC in rats, but not in clinical studies, therefore it will allow informed decision making about recreational use by several countries. This motivates further investigations from this population and to add other exposition biomarkers in order to correlate them with specific concentrations of the agent in biological samples.

CL 26- TRACE METAL LEVELS IN SERUM AND URINE OF A POPULATION IN SOUTHERN BRAZIL

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Introduction: exposure to heavy metals, either acute or chronic, can lead to impairment of human health, being a matter of concern to health agencies. Large scale studies which determine heavy metal concentrations in organic matrices of a specific population are required for proper evaluation of the exposure profile of said population, and serve as basis for further exposure assessments and other toxicological studies. While many such surveys have been conducted with different populations worldwide, Brazil falls short on the amount of studies attempting the same. **Objective:** this work aimed to evaluate serum and urine concentrations of several trace heavy metals of a not directly exposed population in southern Brazil in order to determine reference values. **Material and methods:** serum and urine samples were obtained from 240 volunteers (175 males and 65 females, age ranging from 18 to 74 years old). Participants that reported occupation and habits that might influence trace metal concentrations were ruled out from the analyses. Levels of lead, arsenic, cobalt, nickel, zinc, manganese, chromium and copper were determined in each sample by means of dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS). Results were statistically analyzed using nonparametric Mann-Whitney's test and Spearman's coefficient correlation test. **Results and discussion:** results for serum and urine heavy metal levels found for male and female subjects are shown in the following order (geometric means): arsenic, cobalt, copper, chromium, manganese, nickel and zinc. Male serum concentrations: 1.153; 0.1501; 891.4; 1.951; 0.5295; 0.6993 and 738.9 µg/L. Female serum concentrations: 1.195; 0.1584; 1401.0; 1.965; 0.5593; 0.8378 and 700.9 µg/L. Male urine concentrations: 12.48; 0.2047; 24.53; 2.217; 0.9352; 1.523 and 228.9 µg/g creatinine. Female urine concentrations: 11.34; 0.2505; 27.01; 1.990; 1.104; 1.475 and 186.9 µg/g creatinine. Mann-Whitney tests comparing results between genders resulted in no significant statistical difference for all metals but serum copper, as concentrations are higher in females than males. Spearman's test correlating age and metal concentrations showed a negative correlation between serum concentrations and age for most metals assessed, but no significant correlation between urine concentrations and age. **Conclusion:** reference values for the assessed population have been successfully established. Further analyses showed no difference between genders (copper being the exception) and a decrease in serum levels as the population ages.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 27- PREVALENCE OF RESPIRATORY SYMPTOMS AMONG FARMERS EXPOSED TO PESTICIDES CONSIDERING SMOKING HABITS

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Introduction: The immoderate use of pesticides in agriculture has been related to several health problems, especially among farm workers. The respiratory system is sensible to these pollutants and can react with various symptoms. Some important confounders (e.g. smoking habits and use of wood stove) have to be considered. The economy of Sao Jose de Uba is based on tomato's crop, which requires too much pesticide. **Objective:** To estimate the prevalence of respiratory symptoms among farmers exposed to pesticides in Sao Jose de Uba and to compare the prevalence between smokers and nonsmokers. **Materials and Methods:** Study subjects were interviewed using questionnaires to evaluate socioeconomic factors, possible confounders, pesticide exposure and respiratory symptoms (cough, dyspnea, tightness or wheezing, rhinitis or allergy in nose). The interviews were conducted during the season (Aug/2014) and off-season (Jan/2015). The prevalence ratio was calculated for every symptoms among smokers and nonsmokers. **Results and Discussion:** 44 farmers (37 men and 7 women) were interviewed and the mean (range) age of participants was 43.1 (18-74) years old. The mean exposure time was 31.4 years with a range of 5-66 exposure-years, showing they were very young (mean 12.4 years old) when started working or helping in the crop. Pesticides were mainly applied twice a week in season, the average hours worked daily was 10.7 (range 7-14) and the family monthly income was up to 2 Brazilian minimum salaries (± US$ 500) for 77.3% of farmers. The wood burning stove was used only by 6.8%. In relation to smoking history, 59% were nonsmokers, 20.5% were current smokers and 20.5% were former smokers. A lower prevalence of symptomatic individuals and respiratory symptoms were observed in off-season, even among nonsmokers. During the harvest, 65.9% reported at least one symptom, while in the off-season only 36.4%. The prevalence (and PR - Prevalence Ratio) of cough crisis was 41% in season and 20.5% in off-season (PR=2). For chest tightness and allergy in the nose or rhinitis, the prevalence were 22.7% in season and 15.9% in off-season (PR= 1.44). The prevalence of wheezing was 15.9% in season and 9.1% in off-season (PR=1.78). For shortness of breath, the prevalence was 11.4% in season and 9.1% in off-season (PR=1.22). If considered only the nonsmokers the PR was 2 for cough crises and allergy in the nose or rhinitis, 3.99 for chest tightness and 3 for wheezing and shortness of breath. **Conclusions:** The high prevalence of respiratory symptoms found during the season in comparison to off-season indicates the sensitivity of the respiratory system to pesticides. The higher prevalence ratio founded when considering only the nonsmokers reinforces that.

CL 28- KIDNEY DISORDERS INDUCED BY SERPENTS OF *BOTHROPS* GENUS IN CHILDREN AND ADULTS NOTIFIED TO THE ASSISTANCE CENTER IN TOXICOLOGY (CEATOX) OF THE STATE OF PARANÁ, BRAZIL

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Introduction: Acute renal failure (ARF) is one of the most serious complications on accidents by *Bothrops* serpents, which can lead to death. Studies suggest that ARF is due to renal vasoconstriction and rhabdomyolysis caused by the snakebite, but also,directly due to proteolytic effect of bothropictoxin on the glomerulus, inducing a nephrotoxicity.The physiological differences between individuals, mainly among children and adults, may contribute to the worsening of the renal symptoms caused by bothropic toxin. **Objective:** Investigate the renal function in response to the bothropic accident in children and adults. **Materials and methods:** For the study, we conducted a survey of cases of bothropic accidents met and/or notified to the Assistance Center in Toxicology (CEATOX) of the State Secretariat of Health of Paraná (SESA/PR) in period from 2010 to 2014. The cases were separated according to age as child (equal or less than 14 years old) or adults (over 14 years)and renal function, kidney parameters, urea and creatinine were analyzed. The data came from the information contained in the individual toxicological occurring case analyzed. **Results and discussion:** In the period of study, 129 cases of brothropic accidents were registered, being 81% of the accidents in adults and 19% in children. Among adults the renal markers, urea and creatinine, changed in 32% and 24%, respectively. In other hand, in children's, weren't detected significant changes in creatinine levels, just in urea levels, that were significant at 10% of cases. Although studies suggest that children tend to be more affected by bothropic toxin, leading to a more severe clinical picture, we can observe that were more cases evolving into a renal damage in adults. Possibly, the smallest change in kidney parameters in children can be attributed to agility in caring for children, associated with inadequate screening of adult patients, which leads to delay in their treatment. **Conclusions:**The analysis of kidney parameters shows that adults are more likely to suffer kidney damage and this fact may be related to a delay in patient care.

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A | R | T
APPLIED RESEARCH IN TOXICOLOGY

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Volume 1 / Suppl. 1 / 2015

CL 29- KIDNEY DISORDERS INDUCED BY SERPENTS OF *BOTHROPS* GENUS IN CHILDREN AND ADULTS NOTIFIED TO THE ASSISTANCE CENTER IN TOXICOLOGY (CEATOX) OF THE STATE OF PARANÁ, BRAZIL

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Introduction: Acute renal failure (ARF) is one of the most serious complications on accidents by *Bothrops* serpents, which can lead to death. Studies suggest that ARF is due to renal vasoconstriction and rhabdomyolysis caused by the snakebite, but also,directly due to proteolytic effect of bothropictoxin on the glomerulus, inducing a nephrotoxicity.The physiological differences between individuals, mainly among children and adults, may contribute to the worsening of the renal symptoms caused by bothropic toxin. **Objective:** Investigate the renal function in response to the bothropic accident in children and adults. **Materials and methods:** For the study, we conducted a survey of cases of bothropic accidents met and/or notified to the Assistance Center in Toxicology (CEATOX) of the State Secretariat of Health of Paraná (SESA/PR) in period from 2010 to 2014. The cases were separated according to age as child (equal or less than 14 years old) or adults (over 14 years)and renal function, kidney parameters, urea and creatinine were analyzed. The data came from the information contained in the individual toxicological occurring case analyzed. **Results and discussion:** In the period of study, 129 cases of brothropic accidents were registered, being 81% of the accidents in adults and 19% in children. Among adults the renal markers, urea and creatinine, changed in 32% and 24%, respectively. In other hand, in children's, weren't detected significant changes in creatinine levels, just in urea levels, that were significant at 10% of cases. Although studies suggest that children tend to be more affected by bothropic toxin, leading to a more severe clinical picture, we can observe that were more cases evolving into a renal damage in adults. Possibly, the smallest change in kidney parameters in children can be attributed to agility in caring for children, associated with inadequate screening of adult patients, which leads to delay in their treatment. **Conclusions:**The analysis of kidney parameters shows that adults are more likely to suffer kidney damage and this fact may be related to a delay in patient care.

Acknowledgements: The support of State Secretariat of Health of Paraná (SESA/PR) and the Western Paraná University Hospital (HUOP/PR).

CL 30- CARDIOMYOPATHY IN THE COURSE OF CARBON MONOXIDE POISONING

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Introduction: The carbon monoxide poisoning (CO) is a common cause of morbidity and mortality worldwide. The main mechanism of toxicity is reported hypoxia ischemia, heart and brain being the main target organs affected. Cardiovascular manifestations reported in the literature include: arrhythmias, pulmonary edema, heart failure and myocardial infarction. **Objectives**: To demonstrate the occurrence of CO -induced cardiomyopathy, which is a new disease entity, and there is growing scientific evidence regarding its epidemiology, pathophysiology and clinical features. **Material and Methods**: We present two cases Cardiomyopathy induced by CO, which occurred during 2013. These two members of a family group consisting of mother (M), 55 years and her children (CH1 and CH2), 25 and 21, respectively. **Results and Discussion**: As the only clinical manifestation presented sensory deterioration at home. On admission to guard presented clinical improvement, remaining asymptomatic during hospitalization. Carboxyhemoglobin concentrations were (M) 46%, (CH1) and 25% (CH2) 27%, respectively. I was yesterday informed ECG within normal limits. Markers, CPK and troponin T ultrasensitive showed abnormal values in M and CH1. The echocardiographic evaluation showed impaired left ventricular systolic function: M mild impairment, CH1 severe deterioration and global hypokinesia. Patients evolved reversing myocardial involvement, it is described in the literatura. **Conclusion**: CO-induced cardiomyopathyis an entity with increasing recognition in the medical community, which should be incorporated between cardiac complications should be considered in this type of poisoning.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 31- NEUROLOGIC MANIFESTATIONS LONGER IN SEVERE LITHIUM INTOXICATION

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Introduction: Lithium is used as a treatment for bipolar disorder. Lithium has a narrow therapeutic range and this between 0.6-1.2 meq / l. Moderate to severe lithium toxicity is characterized by altered mental status, tremors, stupor, convulsions, coma, hyperreflexia and arrhythmias. Prolonged exposure of the central nervous system to toxic levels of lithium may cause permanent damage. **Materials and Methods**: The following case is presented, a man of 53 years old admitted to the emergency room by stupor, tremors, fever and oliguria. The patient suffering from bipolar disorder treated with alprazolam, quetiapine and lithium carbonate controlled release 450. **Results and Discussion**: The patient presented with stupor, generalized tremor, hyperreflexia, clonus. Vital signs: hyperthermia, respiratory rate of 12 breaths per minute, peripheral oxygen saturation (SpO2) of 92%, heart rate of 110 beats per minute and blood pressure of 100/60 mmHg. The electrocardiogram (ECG) showed prolonged QTc RS with 0.48 ms. Laboratory tests showed: 17,310 white blood mm3, hemoglobin 15 g / dl, urea 110 mg / dl, creatinine 3 mg / dl 5000 CPK, sodium 135 mEq / L, potassium 4.5 mEq / l lithium 12 mEq / l . Arterial blood gas indicated severe metabolic acidosis. Computed tomography of the brain, chest radiography and abdominal ultrasonography were performed showed no abnormalities. The patient was transferred to the Intensive Care Unit, endotracheal intubation was done with connection to mechanical ventilation, parenteral hydration and initiation of hemodialysis. **Conclusion**: With the start of hemodialysis a gradual lowering of lithium took 72 hours to normalize was evident. Finally the patient was extubated but the patient remained with neurological manifestations such as cognitive impairment, myoclonus and tremors throughout the hospital until he was transferred to a rehabilitation clinic. The suspicion of poisoning was confirmed by the family

CL 32- HEALTH BELIEF MODEL: APPLICABILITY ON CHILD POISONING PREVENTION

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Introduction: The child poisoning are serious chemical emergencies and the reasons their caregivers use its best efforts to prevent them are anchored in environmental and psychosocial factors. To find effective intervention strategies in the prevention of childhood accidents, the problem is approached from different theoretical perspectives. Increasingly, the focus of research and interventions shifted from environmental factors to enhancing the role of behavior and psychosocial factors in the causes and prevention of accidents, and understand how child poisoning brought changes in the behavior of households, subsidizes nursing for the preparation of actions on health recovery and prevention on (re) poisoning. **Objective**: To discuss the applicability of the Health Belief Model to assess preventive behavior of families after hospital discharge of children with severe intoxication. **Methodology**: Theoretical and reflective study, with documentary analysis and literature review, built on reading documents about the MCS and prevention in health and nursing, in electronic databases Virtual Health Library, Scielo, Lilacs and Medline, and additional sources - books, theses and dissertations, search conducted in May 2014. **Discussion**: The Health Belief Model has been approached by different interpretations in the education, health and nursing field, in particular as referential to explain human behavior in addition to health-disease process and helps in understanding changes and maintenance of behavior and research benefits and barriers to adoption of preventive measures. It was one of the first models that fit the theory of behavioral sciences to preventive models in health and it is applied to a variety of situations to understand the behavior in response to health. It considers that health decision-making is encouraged by the perception of susceptibility and severity or seriousness of the injury, by the perception of preventive activities benefits to protect from new episodes of injury and by the individual and social barriers to behavior change. Based on this theoretical framework, this study discussed the following units of analysis: Contextualizing the serious childhood poisonings; The Health Belief Model in disease prevention; and The Health Belief Model in preventing childhood poisoning. **Conclusion**: A theoretical and methodological approach studied facilitates the identification of self-care deficits within the family and the establishment of the support needs of families, is empirically accessible, easy to apply and allows the achievement of projected results.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 33- SUICIDE WITH ELECTRONIC SMOKING DEVICE: CASE ATTENDED AT A TOXICOLOGICAL ASSISTANCE CENTER

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Introduction: Smoking is considered a public health problem, and its practice is restricted by the association for developing multiple chronic conditions. Clinical studies point the nicotine as the main agent to develop tobacco dependence. With legal restrictions, the tobacco industry produced a way of administering nicotine without burning tobacco, the so-called E- cigarettes or electronic cigarettes, a motley collection of electronic smoking devices - DEF, which vaporize liquid nicotine in a similar to cigarette tube. In Brazil the marketing of DEF is prohibited by RDC 46/2009 - National Health Surveillance Agency. **Objective:** To describe a case of attempted suicide by ingestion of liquid nicotine present in electronic smoking device. **Methodology:** Documental study, in the case study modality, with data from epidemiological chart accessed in a center of information and toxicological assistance. **Clinical case:** 21 year-old male, college student, single. Due to family and affective conflicts, ingested undetermined amount of liquid nicotine from electronic smoking device associated with alcohol at his residence. He was admitted in a hospital at the emergency department in cardiac arrest (reversed), bilaterally mydriatic and not reactive pupils, with hypotention. There was no reliable information about the time of intoxication, making it impossible to recognize the progression of the clinical condition at home. It was performed as a precaution, gastric lavage and administered activated charcoal. Transferred to the intensive care unit, he evolved into critical clinical condition, under intermittent mechanical ventilation with positive pressure, sedation, infusion of vasoactive drugs, atropine (anisocoria and miotic pupils), antibiotic therapy (hyperthermia, pneumonia), catheter deployment to monitoring intracranial pressure and intensive nephology interventions. The patient evolved into a “not cognitive syndrome”. Family denied a previous suicide attempt and psychiatric comorbidities. **Discussion:** Attempted suicide by ingesting plant pesticide, tobacco alkaloid, high toxicity when ingested, which resulted in severe intoxication. Absence of major risk for suicide factors - history of suicide attempts and mental disorder - setting the act as " opportunity " for easy access and prior information about the toxicity of the product, which was present in illegal marketing of the device in Brazil but wide range on social networks and sales sites on the internet. **Conclusion:** To alert to the risk of increasing cases , and the uniqueness - tobacco derivative used for suicide - and clinical severity of the events , and the need to reinforce measures to fulfill the legal requirements.

CL 34- HYDROXOCOBALAMIN: NATIONAL AVAILABILITY FOR FIRST RESPONDERS

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Smoke inhalation is the most important cause of fire-related morbidity and mortality. Fires in confined spaces may easily induce systemic poisoning bay a mixture of irritant and asphyxiating gases. Those fire victims patients with suspected airway burns are assumed to have carbon monoxide poisoning , however , there is increasing evidence that cyanide inhalation is at least as important as carbon monoxide when synthetics polymers are burned. Since Bismuth C et al research in the 90s cyanide has been recognized as crucial for fire victims mortality , and Hydroxocobalamin has been considered as an antidote for cyanide poisoning . There are recent European and North American evidence based and consensus treatment protocols in which Hydrocobalamin is considered a first line – first responders treatment for fire victims. Lesson learned after recent regional confined spaces fires (discos and night clubs) have put in evidence that Hydroxocobalamin is neither easily, not commercially available for the first responders in South America Uruguay Poison Center , in collaboration with national authorities , is development a protocol which include an algorithm of decisions, and appropriate availability of Hydrocobalamin for fire related smoke inhalation. The objective of our work is to regionally share the process of this protocol development and the crucial steps that are being faced in order to get available an antidote that need to be administered immediately .

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 35- CARDIOVASCULAR EFFECTS OF CHRONIC COCAINE USE IN YOUNG

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Introduction: Cocaine is the second illegal drug most consumed in America and this situation has a higher prevalence in America (1,4%) than in the entire world. Cocaine hydrochloride soluble form could be sniffed and injected and cocaine paste base, lipophilic could be smoked. Cardiovascular effects have been described in association with cocaine use but the impact in the short/medium-term chronic use of hydrochloride cocaine and cocaine base paste in the arterial system in young subjects has not been studied. **Objetive:** Characterize the cardiovascular effects of chronic cocaine use in young and its relation with cocaine use intensity. **Materials and methods:** Toxicologic assessment was made. Cardiac performance was evaluated by exercise stress/rest 99mTc-MIBI gated myocardial perfusion SPECT (MPS). End diastolic (EDV) ,systolic volume (ESV) and left ventricular ejection fraction (EF) were calculated using Cardiogam software. Correlation between functional MPS parameters and use characteristics was analyzed. Common carotid artery intima-media thickness (CIMT; B-Mode echography), aortic pulse wave velocity (PWV, Applanation Tonometry) and endothelial function (Flow-mediated dilation, FMD) were quantified. Early Vascular Aging (EVA) was evaluated considering the difference between AA and chronological age. Obtained values were compared to reference value. **Results and discussion:** Thirty-one patients were assessed, Mean Age 28,8 years; 29 male. Six patients had ischemia (SDS=3-5). The mean cocaine use was 11,1 years and 11,6 grams per week . EF was reduced (53±7% vs. 59±8%; p=0,003, t test) while both ESVi (34±7ml/m2 vs. 21±9 ml/m2; p<0,001) and EDVi (75±9 ml/m2 vs. 49±13 ml/m2; p<0,001) were increased in cocaine users. Strong linear relationship was found between EF and ESVi (r=-0,80, p<0,001) . No relationships were found between functional MPS parameters and dose or time of cocaine use. Impaired arterial parameters were found: CIMT (62%), PWV (27%) and FMD (35%). When arterial changes were jointly analyzed an AA of 37.1±8.4 years was obtained which resulted in an EVA of 8.3±6.2 (range: 3-24) years. **Conclusions:** Cocaine users showed asymptomatic post-stress ventricular dysfunction and subclinical arterial structural and functional detrimental changes associated with an increased cardiovascular risk. Variables such as dose, time and frequency of used in the cardiovascular changes associated with cocaine abuse should be investigated in larger series.

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CL 36- SMOKED COCAINE: MEDICAL AND SURGICAL COMPLICATIONS

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The main smokable cocaíne in Uruguay is Cocaine Paste Base (PBC), and its health impact is very importtant. Hospital consults are due to intoxications, withdrawal, and medical or sugical complications. **Objetive**: To acknowledge the main health problems and the users abuse profile of PBC in a university hospital . **Methodology**: This is an observational retrospective and descriptive study. We analized clinical cases of PBC users. 78 patients were evaluated, all of them had dependence diagnosis; 9 cases couldn`t be evaluated. Of the total cases 58 were male patients and 20 females (14 obstetric cases); the age mean was 27 years (range16 – 56).Multiple drug consumption was mostly found (74 patients). **Results:** The most frecuent consume devices were different materials handmade pipes. The reasons for hosptialization were several, principally infections (28 cases) and violent injuries (28 cases); intoxication and overdose were the cause in 14 cases. The main infectious diseases were HIV in 10 patients, complications of intravenous drug abuse (3 cases) and Hepatitis C (4 cases). Three patients were admitted in the National Burn Center. Most patients were hospitalized in surgical areas (28), followed by medicine and emergency areas. Primarily the abuse profile was of PBC exclusively in handmade pipes (66 cases); only 8 also consume cocaine and 2 smoke PBC in cigarettes associated with marijuana (“basoco”). Most patients had between 5 and 9 years of drug abuse history; and 27 wanted to cease the drug abuse. **Conclusions**: there is a higher demand of hospitalization due to medical or surgical complications among PBC users. The clinical presentation is multiple; mostly infectious and traumatic diseases. It is also very important the toxicological cause that includes intoxication and withdrawal. It is very important to have a specialized program to treat these patients. Continuous investigation will add more knowledge to this complex issue.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 37- DIAGNOSTIC IMAGING IN CLINICAL TOXICOLOGY

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Some toxicologic emergencies, and even chronic toxic effects of several substances, can be diagnosed by imaging methods. Many imaging modalities can be used according to the medical care setting where the patient is being seen, or according to the kind of substance involved. Around 30 substances, including some metallic elements, can detected or produce body alterations visualized by some of the imaging modalities currently available. Some selected cases of venomous animals bites can also be better managed using imaging methods. Conventional radiography (analogue or digital) is readily available in emergency rooms and can surely be useful in some acute intoxicated patient management. Computed tomography can add information to conventional images in an emergency setting, as can ultrasonography, magnetic resonance imaging, and nuclear scintigraphy. The two main purposes of using imaging diagnostic methods in toxicology and toxinology are: 1) visualizing and identifying the xenobitoic, or 2) visualizing its effects. The first objective is accomplished when substances or elements are radiopaque. The radiopacity is dependent of the substance density and the atomic number of the elements it contains. Most of the toxic effects can be seen in the lungs, followed by the brain, bones and liver, depending on the pathophysiology of the specific intoxication. The objective of the talk is to highlight the importance of the use of diagnostic imaging in the management of intoxications, presenting selected clinical cases as examples, discussing the characteristics of the intoxication involved in the cases, and the several imaging methods available, or indicated, to each clinical presentation.

CL 38- EPIDEMIOLOGICAL PROFILE OF INTOXICATION EXOGENOUS ON A PARAÍBA STATE REGION

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Introduction: exogenous intoxication is the manifestation through signs and symptoms of the harmful effects produced in a living organism resulting from its interaction with some chemical. Nowadays, with the increasing inclusion of chemicals on the market and easy access to drugs and alcohol, there was a very significant increase in accidents related to this type of intoxication, resulting in a health public problem. By the context noticed themselves to the absence of studies about this theme in Paraíba, thus motivating for the preparation of this work. **Objectives:** Describe the epidemiological profile of exogenous intoxication in 24 municipalities attended the 6th Paraíba State Health Management in the years from 2009 to 2015. **Materials and Methods:** This is a applied study, retrospective, analytical approach with a qualitative and quantitative manner. Data were collected in the National Notifiable Diseases Information System (SINAN) for sanitary and hospital officials in the city of Patos-PB, headquarters of the 6th of Paraiba State Health Department, in January 2009 to June 2015. **Results and Discussion:** they totaled only 35 cases of exogenous intoxication between the years 2009 to 2015, only being specified cases of the year 2015 for the 6th Health Management of the State of Paraiba, because until the year in question had not been implemented a system computerized for the reporting of cases this management, knowing only the amount by sex. Between the years 2009 to 2013, only two cases have been reported, both in males. In 2014, there were 29 reported cases, 17 in females and 12 in males and by June 2015 4 cases were reported, 3 in females and 1 male, these cases filed in 2015, 2 were harsh: fuel and caustic soda; 1 oral drug and alcohol intoxication 1. The highest rates of notifications were in the female sex people, leaving 57% of cases. This data can be related to the fact that women work more often with chemicals for domestic cleaning excessive and indiscriminate use of these products, ignorance of the dangers along with the lack of a proper handling. It is noteworthy that both sexes are at risk of all kinds of exogenous poisoning. **Conclusion:** With the results, it is worth emphasizing the need for greater control through educational measures for the sale of chemicals, drugs and alcoholic beverages, and a preventive action of women for the proper use of these products in relation to domestic cleaning, to modify this epidemiology.

A | R | T
APPLIED RESEARCH IN TOXICOLOGY

ISSN: 2359-4721
Volume 1 / Suppl. 1 / 2015

CL 39- ACUTE ALCOHOLIC INTOXICATION IN A CHILD OF 11 YEARS

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Introduction: The clinical manifestations of acute alcohol intoxication, depend on the concentration of alcohol in blood. Its pharmacological action is the central nervous system depressant. Most acute ethanol poisoning are intentional, voluntary ingestion by abstainers and / or chronic alcoholics. Less often accidental ingestion in children occurs due to the presence of home use products containing alcohol. In recent years it has increased the intentional use among children and adolescents. In our hospital during 2014 within the samples processed with alcohol positive (> 0.5 g / L) 47.4% are under 18 years old. **Objectives**: To describe the clinical consequences of acute alcohol intoxication. Highlighting easy access to alcoholic beverages both inside and outside the home. **Materials and Methods**: Dosage of alcohol in whole blood with the technology of radiative energy attenuation (REA, Abbott); dosage of drugs of abuse in urine with the technology of fluorescence polarization immunoassay (FPIA Abbott). **Case report**: Male patient 11 years old with no medical history. Cause for medical consultation, head trauma with loss of consciousness, secondary to intentional ingestion of ethyl alcohol (beer) and benzodiazepines (clonazepam) unknown quantity. He enter to the guard the hospital with ataxic and dysarthria. He is internal to control blood glycemia, vital signs, parenteral hydration it indicated with dextrose. Alcohol and dosage of drugs of abuse in urine with positive results (Alcoholemia 3.3 g / L Metabolites in Urine: no detectable cocaine, benzodiazepines and cannabinoids were positive). **Conclusion**: Easy access, imitative behavior with the adults and school leavers, are some risk factors among children beginning to "experiment" this kind of abuse, with serious potential risk due to the ingestion of alcoholic beverages, benzodiazepines and drugs of abuse. We stress the importance of toxicological laboratory determinations oriented towards substance abuse.

CL 40- EVALUATION OF ANALGESICS IN POISONING CASES AND ANTI-INFLAMMATORY IN A HOSPITAL EMERGENCY AND PUBLIC EMERGENCY NETWORK OF MINAS GERAIS

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introduction: Poisoning arising from painkillers and anti-inflammatory is a major public health problem and has been linked to an increase in the inappropriate use of these drugs. This misuse may result in turn to increased mortality and health. **Objective**: To evaluate the cases of poisoning by analgesics and anti-inflammatory in an emergency hospital and emergency public of the state of Minas Gerais. **Material and Method** :We conducted a retrospective, descriptive, from intoxication cases seen and recorded from January to December 2014 in a tertiary state hospital. For data collection were used internal records of Toxicology Unit of Hospital João XXIII. The variables used were: identification, month, age, gender, exposure time, circumstance, agent, dose ingested, antidote, hospital stay, conduct and evolution. **Results and Discussion**: Obtained 81 cases of poisoning caused by anti-inflammatory and analgesic within them 38.67% for males and 61.33% for females. It was observed that the predominant age group was adult-youth, aged 20-29 years (29.63%), followed by the age group 15-19 years (19.75%) and (18.52%) of patients they were aged 30-39 years. Regarding the time of exposure to drugs (22.21%) of contaminated arrived at the hospital up to two hours after ingestion of the toxin (34.58%), arrived with over two hours of elapsed time of exposure, and (43.21%) of those exposed were ignored as this data. In the sample,% 56.69 requiring no antidote, and the remainder (43.31%), 18.51% active carbon, 20.99% n-acetylcysteine, 3.70% and association of activated charcoal plus n-acetylcysteine .Given the circumstances that led to the poisoning, 61 people attempted self-extermination, 14 accidental, 4 were error management and 2 self-medication. There was a need for guidance and awareness of the population about the risks of exposure of these drugs and the role of industry in manufacturing (eg, packaging resistant children). Of the total cases, 39.51% required hospitalization, of these, 13.58% were hospitalized over a period of up to two days, 24.68% three to five days and 1.23% over five days. Acetaminophen was the analgesic responsible for the largest number of cases, representing 62.96%. From the anti-inflammatory drug (NSAID), ibuprofen 24.69%, followed by diclofenac, nimesulide, and aceltisalicílico acid (respectively 17.28%,11.11% and 7.40%). **Conclusion**: This study provides important information for health education and knowledge of the causes that lead poisoning.

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