EUROTOX 2016 - Late Breaking Abstracts

LBA02 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Repeated Dose Inhalation Toxicity Study Of Fenbutatin Oxide Formulation In Wistar Rats

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Vendex 50 WP (Fenbutatin oxide) is widely used for agriculture applications. To assess hazard potential of air-born Vendex 50 WP, the long-term inhalation study in male and female rats was performed, according to the OPPTS test guidelines. The rats were exposed to 0.002 mg a.i./L air (G2), 0.008 mg a.i./L air (G3), 0.016 mg a.i./L air (G4 and G6) for 6 hour/day, 5 day/week for 4 weeks respectively, in a 28-day study. A variety of biological clinical chemistry, hematology, urine analysis, organ weight and histopathology were examined. In the present study, the treatment of Vendex 50 WP was associated with decrease in prothrombin time, increase in absolute and relative weights of lungs, increase in volume and pH of urine and decrease in specific gravity of urine at 0.008 mg a.i./L air (intermediate concentration) and 0.016 mg a.i./L air (high concentration),in the absence of any histopathological findings in organs associated with these findings, which were considered as an adaptive response to test item.. Therefore, the No Observed Adverse Effect Concentration (NOAEC) is 0.016 mg a.i./L air, when administered, through inhalation, up to 28 days, to Wistar rats under the procedure and conditions followed in the present study. Whereas, the No Observed Effect Concentration (NOEC) of Vendex 50 WP is 0.002 mg a.i./L air.

Keywords: Fenbutatin oxide, Lungs, NOAEC

LBA03 [Accepted:Poster Presentation] [Alternative Animal Models]

Study for the Selection of Appropriate Vehicle in Bovine Corneal Opacity and Permeability (BCOP) Test

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Bovine Corneal Opacity and Permeability Assay (BCOP) is an ex-vivo assay, which is used to assess the eye irritation potential of chemicals. The BCOP assay is accepted by several regulatory agencies for the identification of corrosive ocular irritants, replacing the rabbit eye test. As per the OECD 437 for eye irritation (BCOP assay), non-surfactant solids are tested as 20% concentration in 0.9% sodium chloride or other solvent that has been demonstrated to have no adverse effects on the test system. However, the limited solubility of some chemicals adds technical challenges in finding a suitable vehicle that would ensure the material's availability to the excised corneas without affecting the test system. In this study, we evaluated four solvents: normal saline, olive oil, tween 20 and propylene glycol. Based on the available classification systems, our preliminary data showed that normal saline, olive oil, tween 20 were predicted as non-irritants, while propylene glycol had an irritating effect on the cornea. To demonstrate the influence of vehicle on the outcome of the BCOP assay for solid materials, we tested a 20% suspension of dicamba prepared in olive oil, tween 20 and propylene glycol. Previous animal tests have reported corrosive effect of dicamba. Our results demonstrated that when mixed in propylene glycol and corn oil, dicamba was predicted to be a corrosive while it was predicted to be a moderate irritant when mixed in tween 20. These results support the need for further investigation of the solvent's influence in the BCOP assay.

Keywords: BCOP, Dicamba, IVIS Score

LBA04 [Accepted:Poster Presentation] [Emerging In Vitro Models]

Consortium for *In Vitro* Eye Irritation Testing Strategy: Short Time Exposure Model

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Assessment of ocular irritancy is an international regulatory requirement in the safety evaluation of industrial and consumer products. The objective of the CON4EI project is to develop tiered testing strategies for eye irritation assessment for all drivers of classification. For this, a set of 80 reference chemicals (38 liquids and 42 solids) was tested in eight *in vitro* test methods. Here, the results obtained with Short Time Exposure (STE) model are shown. The primary aim of this study was an evaluation of the performance of the test method to discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labeling for inducing serious eye damage (Category 1). In addition, the predictive capacity in terms of *in vivo* driver of classification was investigated. In a second step, it was investigated if STE can be used as part of a tiered-testing strategy for eye irritation assessment when assessing chemicals that fit the applicability domain.

For the STE method, the accuracy in identifying Cat 1 chemicals was 61.3% with 23.7% sensitivity and 95.2% specificity. Excluding non-qualified results did not affect the ability to correctly identify Cat 1 chemicals (accuracy 61.2% with 26.9% sensitivity and 100% specificity). The accuracy of the STE test method to identify No Cat chemicals was 72.5% with 66.2% sensitivity and 100% specificity. Excluding non-qualified results improved the predictivity (accuracy 87.8% with 85.4% sensitivity and 100% specificity).

This research is funded by CEFIC-LRI. We acknowledge Cosmetics Europe for their contribution in chemical selection.

Keywords: CON4EI, STE, ocular irritation

LBA05 [Accepted:Poster Presentation] [Emerging In Vitro Models]

A Human Brain Microphysiological System derived from iPSC for Parkinson's disease drug screening

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Human in-vitro models of brain neurophysiology are needed to investigate molecular and cellular mechanisms associated with neurological disorders and neurotoxicity. We have developed a reproducible iPSC-derived human 3D brain microphysiological system (BMPS) containing mature neurons and glial cells (astrocytes and oligodendrocytes), which reproduces the topology of neuronal-glial interactions and connectivity. A BMPS, derived from healthy donors or patients, differentiates and maturates over 8 weeks and contains the critical elements of neuronal function, synaptogenesis, neuron-neuron (e.g. spontaneous electric field potentials) and neuronal-glial (e.g., myelination) interactions, which mimics the microenvironment of the central nervous system. We have used the BMPS to establish a model for evaluating neurotoxicants linked to pathogenesis of Parkinson's disease (PD). The neurotoxic effects of rotenone or 1-methyl-4-phenylpyridinium (MPP+) were evaluated in the BMPS model system, in which the altered expression of PD-related genes was observed that recapitulates the hallmarks of PD pathogenesis linked to toxicant compounds. Thus, the BMPS provides a suitable and reliable model to investigate neuron-neuroglia functions in neurotoxicology or other pathogenic mechanisms.

Keywords: iPSC, Parkinson's disease, 3D brain model

LBA06 [Accepted:Poster Presentation] [Food Safety / Food Allergies]

Lead and mercury accumulation in edible parts of vegetables collected from Markazi province/Iran and the survey of bio toxic effects

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Vegetables are important protective foods and highly beneficial for the maintenance of health because they contain valuable food ingredients like minerals, fibers, vitamins and supply elements. Heavy metals like mercury (Hg) and lead(Pb) are well known metals to creating harmful effect on human's health. Vegetables can absorb metals from soil and accumulate them in their leaves. Two perennial plants which can gather metals in tissues are Tarragon and Mint. These vegetables are commonly used in human diet.
The aim of this study was to determine the amount of mercury and lead in two vegetables namely Mint and Tarragon.
Material &Method
Green leafy parts of Tarragon and Mint in different agricultural sites of Markazi province/lran and also soil around them were gathered. The amount of heavy metals were measured by using ICP-OES.
Result & Discussion
The results (mean ±SD) show Tarragon can absorb high amount of mercury and lead in leaves with 56.147±17.30 and 1055.53±73.90 The concentration of these metals in Mint was 49.0±29.72 and 1017.11±113.40 respectively. After analyzing the soil, the results for these elements, Hg and Pb, were 50.056± 16.25 and 4993.83±1287.8 respectively. The survey of transfer factor shows vegetables can absorb high amount of Hg from soil while these two plants uptake less concentration of Pb in their green leaves. It can be inferred that plans have ability to accumulate toxic metals. It is necessary to measure the amount of toxic elements to reduce biotoxic effects on human and the mechanisms of their biochemical activities.

Keywords: heavy metals, vegetables, bio toxic, soil, transfer factor

LBA07 [Accepted:Poster Presentation] [Regulatory Toxicology]

Possible consequences of various options to identify and categorise endocrine disruptors: case study with natural substances

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Substances with endocrine disrupting properties are under special consideration in European Union regulations. In 2014 the European Commission (EC) published a roadmap which presented four possible options for identifying endocrine disruptors (ED). An impact assessment of the consequences of the four EC options has been performed by JRC which regarded about 700 example substances mainly regulated under the Plant Protection Product Regulation, Biocidal Product Regulation and the REACH Regulation. However, natural substances were usually not considered in this screening activity. Therefore, Cefic (European Chemical Industry Council) and ECPA (European Crop Protection Association) initiated and sponsored a screening activity to assess the consequences of the EC options and an additional industry approach for classification of natural substances. In a first set four substances (genistein, caffeine, vitamin D3, sucrose) were evaluated. This screening activity primarily focused on EATS (estrogenic, androgenic, thyroid and steroidogenic) mediated effects. Although it does not replace a full weight of evidence substance evaluation the screening demonstrated that some natural substances present inter alia in daily food would most certainly be identified as endocrine disruptors (genistein, caffeine) due to EATS mediated effects. Vitamin D3, which is physiologically produced in the human body but also exogenously added, would be identified as endocrine disruptor due to the interaction of its active metabolite cholecalciferol with the parathyroid hormone. Only for sucrose no evidence for endocrine disrupting activity could be identified.
The detailed results of the screening evaluation obtained under the different options will be presented and limitations will be discussed.

Keywords: endocrine disruptor, natural substances, sreening

LBA08 [Accepted:Poster Presentation] [Regulatory Toxicology]

Read-across based on metabolomics in vivo: a phenoxy carboxylic acid herbicides case study

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Grouping of chemicals and read-across is a way to provide safety information while keeping animal testing to a minimum. Here we show how in-vivo-metabolomics can support chemical grouping and read-across from a biological perspective. MCPP was selected as the target-substance, MCPA and 2,4-DP as source-substances. For all substances, plasma-metabolome data from 28-day repeated-dose toxicity studies was available in the BASF data-base MetaMap®Tox.
The 28-day metabolome evaluation of source- and target-substances indicated liver and the kidney as the target-organs. Using the information of the 90-day study of 2,4-DP (best source-substance based on metabolome data), the predicted effects of MCPP would have been decreased body weight gain, liver and kidney as target-organs and a moderate reduction of red-blood parameters at 2,500 ppm. The NOEL would have been expected to be below that of 2,4-DP (i.e., <500 ppm) and more likely in the range of that of MCPA (i.e., ≥150 ppm).
From a qualitative view-point, these predictions are very similar to the results of the actual 90-day study in rats performed with the target-substance. From a quantitative point of view, the predicted NOAEL of 150 ppm is in the range of the actual study (NOEL 75 ppm, NOAEL <500 ppm). Consequently, the 90-day rat toxicity study of the target-substance could have been waived and substituted by the 90-day results of 2,4-DP. This case study was presented and discussed at the ECHA-TSWS on New Approach Methodologies in Regulatory Science. The overall assessment confirmed that metabolomics could well serve as a biological basis for read-across.

Keywords: metabolomics in vivo, grouping, read-across, REACH, MetaMapTox

LBA09 [Accepted:Poster Presentation] [Genotoxic Impurities]

Mutagenicity studies of herbicide imazamox in the mice bone marrow micronucleus assay

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Our laboratory of mutagenesis is conducting research on the genotoxicity of chemicals in the battery test systems. Studies carried out in accordance with the requirements of GLP. One of the metods used by the laboratory is mammalian *in vivo* erythrocyte micronucleus test (OECD 474).
We explored 4 samples of active ingredients of herbicide imazamox from different manufactures. This samples were generic and had various percentage. Mutagenicity studies examined on CD1 healthy young adult mice, males, which weight was 18-20 g and acclimated to the laboratory conditions for at least five days.
The test substance was administered as an aqueous emulsion, once orally. Every samples studied in three doses 2500, 250, 25 mg kg-1 and accompanied with positive and negative controls. The time of exposure was 24 hours.
As a result of studies of all of testing samples of imazamox mutagenic effect was not found. However, in high concentrations of test chemicals, it was noted increase of frequency of micronucleus in polychromatic erytrocytes was not significant in comparison to the negative control. The relationships between the frequency of micronuclei, the percentage and impurities is not observed.

Keywords: imazamox, pesticides, mutagenicity, bone marrov, polychromatic erytrocytes, in vivo

LBA10 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Dietary and operator risk assessment of indaziflam 19.05% SC with ADI and AOEL established in Korea

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Toxicity evaluation and establishment of reference dose for indaziflam was conducted with acute toxicity, short term, long term, carcinogenicity, reproduction, teratogenicity, and genotoxicity test. Oral absorption was over 80 %. Category of Acute toxicity was 4. In 90-day dog study, axon degeneration was observed, then the NOAEL was 7.5 mg/kg/day. In 1-year dog study, axon degeneration was observed, then NOAEL was 2 mg/kg/day. There was no carcinogenic, and reproductive/teratogenic effect. The lowest NOAEL was 2 mg/kg/day in 1-year dog study, then the Korean ADI was established as 0.02 mg/kg/day. The most appropriate NOAEL among short term studies was 7.5 mg/kg/day in 90-day dog study then the Korean AOEL was established as 0.075 mg/kg/day. Indaziflam 19.05% SC was used in apple and mandarin. Dietary risk assessment was conducted with maximum detected quantity, intake dose, and ADI. Daily intake of apple and mandarin in Korea was 21.09 and 23.28 mg respectively and maximum detected quantity was 0.01 and 0.01 mg/kg respectively, and MRL was 0.05 and 0.05 respectively. Total daily intake of indaziflam was 0.000444 mg in maximum detected quantity and 0.00222 in MRL. Acceptable daily intake was 1.1 mg (55 kg bw). The total daily intake compared with acceptable daily intake was 0.202 % in MRL, then risk was low. Operator risk assessment was conducted in application for apple. The operator exposure was 0.00921 mg/kg/day in non PPE and 0.00215 mg/kg bw/day in impermeable coveralls. As a result, TER was 8.15 and 34.83 respectively with/without PPE, then the risk was low.

Keywords: ADI, AOEL, risk assessment

LBA11 [Accepted:Poster Presentation] [Regulatory Toxicology]

Validation of a Cytotoxicity Bioassay Kit Based on Assay Ready Frozen Cells for the Biocompatibility Testing of Medical Devices

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Medical devices have to be tested for their biocompatibility before the products are released for use. The protocol, how the cytotoxicity of medical devices is assessed in vitro is regulated in the ISO 10993 guideline part 5. Preferably a murine fibroblast cell line L-929 from an exponentially growing culture is used and incubated with extracts or parts of the medical device.
The continuous availability of the cells at a consistently high quality is essential to perform the assay in a reliable and reproducible manner. To avoid cell culture related variances, we evaluated a cytotoxicity bioassay kit which includes validated aliquots of assays ready L-929 cells. The cells have been cryopreserved at a highly viable and functional state and can be instantly used in the assay without any prior cultivation.
We have tested the kit with reference extracts known to be non-toxic, slightly toxic, toxic or strongly toxic and compared the results with our internal procedure. In addition, the robustness of the assay when performed by different users and the reproducibility of the kit from batch to batch was investigated. We demonstrate that the use of assay ready L-929 cells as they are provided within the kit are a valid alternative to cells which had been cultivated before.

Keywords: medical device, biocompatibility, cytotoxicity, ISO 10993, L-929, assay ready cells

LBA13 [Accepted:Poster Presentation] [Biomarkers]

Forensic Identification of Mixed Seminal and Vaginal Fluids by using microRNA Markers

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Identifying the origin of the body fluids left at a crime scene is important for crime scene reconstruction. Recently mRNA can be used for identification of the biological body fluids, but the mRNA is prone to degradation by biological environmental factors such as UV, heat. The microRNA belongs to a class of small RNA containing 18-25 nucleotides and they are resistant to degradation, also the microRNA markers are almost specific to each body fluid. In this thesis study we identified mixed seminal and vaginal fluid samples through detection of miRNA markers using RT-PCR, we determined that these markers (miRNA 124a and 135b) are specific to the vaginal and seminal fluids respectively, more over we determined that storage for 3 months didn't effect the presence of these markers in the vaginal and seminal fluid samples.

Keywords: Vaginal fluid, Seminal fluid, miRNA 124a, miRNA 135b, RT-PCR.

LBA14 [Accepted:Poster Presentation] [Nanomaterials]

Evaluation of the potential health risk of silver nanowires via dermal exposure

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Silver nanowires (AgNWs) have a large range of anticipated nanotechnology applications such as flexible electronic devices (touchscreen displays), biomedical diagnosis and intelligent fabrics. However, there are currently very few studies of the potential hazards of AgNWs and none investigated the dermal route of exposure. We have then performed AgNW uptake and cytotoxicity studies on human primary keratinocytes and reconstructed epidermis.
Briefly, we studied AgNWs of two length coated with PVP. Primary keratinocytes were exposed to AgNWs and AgNO3. Cytotoxicity was investigated by MTT and NRU assays. Penetration of Ag NW in cells was investigated by SEM and confocal microscopy. Data obtained showed a moderate toxicity of AgNW, which was lower than silver ion but not due to silver ion release in the medium. Ag NW were efficiently internalized by cells and found to be persistent. Also, to be closer to physiological conditions, we used reconstructed human epidermis (epiCS, CellSystems) that were exposed to AgNW. Cytotoxicity was assessed by MTT assay and skin section were also performed and analyzed by TEM. No cytotoxicity was observed even after 72h of exposure and penetration inside the epidermis was not observed.
These results suggest an uptake and moderate toxicity of AgNW in keratinocytes but efficient protection of the stratum corneum in 3D epidermis against AgNW exposure. But several parameters have been shown to promote nanomaterial penetration into viable skin that would need to be further investigated in the case of AgNW skin toxicity and penetration.

Keywords: Silver, Nanowire, skin, toxicity

LBA15 [Accepted:Poster Presentation] [Alternative Animal Models]

Comparative characterization of the glutamatergic system in primary hippocampal neurons and three-dimensional cultured human neuroblastoma cells

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The development of neurotoxicity studies is hampered by the lack of suitable in vitro models to evaluate chemicals induced toxicity on CNS. To counteract this deficiency and to comply the directive asking for animal use reduction, we investigated whether combining three-dimensional human neuroblastoma SH-SY5Y cells cultured in extracellular matrix gel with several neuro-differentiating factors might represent an alternative to primary rat hippocampal neurons. In particular, we focused on the characterization of the glutamatergic system, due to its key role in the progression of neuronal degeneration and cognitive deficits and to literature evidence supporting the expression of glutamatergic receptors in SH-SY5Y.
Three-dimensional cultured SHSY5Y were evaluated for the expression and function of receptors cardinal for neurotoxicity, i.e. NMDA and AMPA receptors, and compared to hippocampal neurons, as reference cells for the glutamatergic system. In particular GluN1, GluN2A, GluN2B subunits of NMDA and GluA1, GluA2 subunits of AMPA receptors were evaluated. Our results show that these subunits, with the exception of GluA2, are faintly expressed in undifferentiated SHSY5Y compared to primary hippocampal neurons. The differentiation process, further reduce their expression. Accordingly, glutamate or NMDA up to 100μM were unable to increase intracellular calcium in SH-SY5Y, while consistent and sustained calcium increase was evident in primary hippocampal neurons at 10 μM. Furthermore, stimulation of differentiated SH-SY5Y by 1mM Glutamate did not induce currents as assess by electrophysiology.
This study shows therefore that three-dimensional cultured SH-SY5Y cells are not an appropriate model to evaluate chemicals affecting the glutamatergic system.

Keywords: glutamatergic system, in vitro model, neuroblastoma cells, primary hippocampal neurons

LBA16 [Accepted:Poster Presentation] [Genotoxic Impurities]

Genotoxicity study of florasulam exposure in micronucleus test *in vitro*

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Potential genotoxic and cytotoxic effects of the generic pesticide florasulam 98 % were investigated using micronucleus assay on primary human peripheral blood lymphocytes *in vitro* in variants with and without metabolic activation (according to OECD 487 guideline in compliance with GLP). Next test concentrations ranging (0,02; 0,07; 0,22; 0,67; 2 *mg/mL*) were defined. Positive controls (cyclophosfamide, mitomicin C, colchicin) for assessing of clonogenic and aneugenic activity were investigated. Negative controls of donor, solvent and S9-fraction were used too. Cytohalasin B was used as a cytokines blocker. As a result, no statistically significant induction of micronuclei in all concentrations and in both S9-variants was seen. Cytokinesis-block proliferation index (CBPI) and replication index (Rl) were calculated in all test concentrations on the basis of the distribution of mononucleated, binucleated and multinucleated lymphocytes. However, evident cytotoxic and cytostatic effects were presented as a in the cytokinesis-block proliferation index (CBPI) was obtained. Inhibition of proliferation after florasulam exposure on human peripheral blood lymphocytes *in vitro* at the high concentration level – 2 *mg/mL* with metabolic activation was demonstrated (57.80 %). As a conclusion that the generic pesticide florasulam was negative for mutations in micronucleus test on human peripheral blood lymphocytes *in vitro*. This results fully equate to review of EPA (2007).

Keywords: generic pesticede, genotoxicity, micronucleus assay, florasulam

LBA17 [Accepted:Poster Presentation] [Clinical Toxicology]

Edible mushroom poisoning

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Amanita ponderosa is an edible and endemic mushroom of south Spain which is easily mistakable with others poisonous species of Amanita. We reported two cases of poisoning after a misidentification of a toxic mushroom as an *Amanita ponderosa*.
Two brothers (20 and 24 years old) were admitted to an emergency department with symptoms of vomit, diarrhoea and abdominal pain. They reported have been collected and eaten some mushrooms three days before. Symptoms had started a few hours after the meal, and as it didn't disappear, they decided to go to a hospital to be treated. Analytic parameters showed hepatic alterations: ALT 2391 U/L and 294 U/L, AST 1476 U/L and 210 U/L, LDH 752 U/L and 235 U/L, respectively.
They were admitted to hospitalization and treated with the specific antidote silibinin (dose 250 mg/6h or 350 mg/6h, in each case) and penicillin G sodium (25 MU/24h).
After seven days, patients had not any symptom and elevated analytic levels had descended: ALT 490 U/L and 39 U/L, AST 41 U/L and 20 U/L, LDH 179 U/L and 145 U/L, respectively, so they were discharged. A month later, they were evaluated by the physician, being analytic levels normalised.
We have observed that similar appearance between some mushroom species can easily cause poisoning if one of these is toxic. In our case, it is thought these two patients were probably poisoned with an Amanita species. Treatment with specific antidote silibinin and penicillin G sodium has shown to be effective in Amanita poisoning.

Keywords: Silibinin, amanita, poisoning

LBA18 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Risk assessment of laureth in cosmetic products

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Laureth-9 belongs to the group of alkyl polyglycol ethers, commonly called alcohol ethoxylates (AEs), and is chemically defined as an AE with an average alkyl chain of 12 to 14 carbon atoms (C12-14) and an ethylene oxide chain of 9 ethylene oxide units (EO9). Laureth-9 has been widely used in the manufacture of personal care products. In Europe, it is most widely used in rinse-off products as a non-ionic emulsifier and co-surfactant, particularly in shampoos and hair conditioners in concentrations up to 4%. It is further used in leave-on products such as body and face creams at levels up to 3%. Currently, the concentration limit of laureth is 2% in the finished cosmetic products. We performed risk assessment for laureth as a cosmetic ingredient. Systemic exposure dosage (SED) was estimated to be 0.12 mg/kg/day. And then no observed adverse effect level (NOAEL) was considered to 37.5 mg/kg/day. The margin of safety (MOS) for laureth in cosmetic products was calculated to be 312.5 based on 37.5 mg/kg/day (NOAEL) / 0.12 (SED). These data suggest that laureth has no risk to human when it is exposed to 2% of laureth in a set of cosmetic products, confirming its safety.

Keywords: Laureth, risk assessment, NOAEL, SED, cosmetic

LBA19 [Accepted:Poster Presentation] [Other]

Method Validation for Quantification of Mesenchymal Stem Cell in Nude Mice Tissues Using Sensitive and Quantitative Real-Time PCR Technique

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Mesenchymal stem cells (MSCs) are one of the most promising therapeutic agents for immune disorders and tissue repair because of their differentiation abilities, trophic and immunosuppressive properties. Nonclinical data on biodistribution of MSCs are considered important and mandatory for clinical therapy approval as the cells have a potential for persistence and differentiation in both target and non-target organs. Quantitative real-time polymerase chain reaction (qPCR) technique is a current gold standard as it is the most sensitive detection mothed for biodistribution of cell therapy products. In present study, a sensitive and quantitative qPCR method for determination of human adipose tissue-derived MSCs in nude mice tissues has been developed and validated for further nonclinical biodistribution study. The validation was conducted using an Alu primer of human specific gene. There were no interfering reacts with the targeted sequence between true positive and negative control samples. The correlation coefficient of MSCs in nude mice tissues was 0.9999 ($1/x^2$ weighted) over a concentration range of 0.005 to 100 ng. The LLOQ and LOD were 0.05 and 0.005 ng/μg, respectively. Also, accuracy and precision were verified by intra- and inter-batch analysis. Thus, the analytical method was confirmed for specificity, linearity, LLOQ, LOD, accuracy, and precision. The human Alu primer used in this study was highly sensitive for detection of human adipose tissue-derived MSCs. In conclusion, qPCR method for biodistribution analysis of MSCs has been successfully developed and validated for further good laboratory practice (GLP) compliant nonclinical biodistribution study.

Keywords: Mesenchymal stem cells, Nonclinical study, Biodistribution, Method validation, qPCR

LBA20 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Risk assessment of a-Damascone in cosmetic products

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a-Damascone has been used in cosmetics as an additive for flavor and also used in body lotion, face cream, eau de toilette, fragrance cream, antiperspirant, shampoo, bath products, shower gel, toilet soap, and hair spray. The cosmetic regulations of a-damascone are specified less than 0.02% in Korea, less than 0.02% except for oral care in Europe but not specified in the United States and Japan. The no observed effect level (NOEL) was estimated to be 0.25 g/kg bw/day, when rats were orally administered a-damascone in 0.25, 0.5, 1.0, 2.0 g/kg for 14 days. 2.0 g/kg dose group of experimental animals is that both experimental day 10 died. Based on this information, systemic exposure dose (SED) and margin of safety (MOS) were calculated for cosmetics. A risk assessment was carried out in cosmetics by no observed effect level (NOEL) / systemic exposure dosage (SED). Thus, risk for a-damascone in cosmetic products was calculated to be 4,166.66 based on 250 mg/kg/day (NOEL) / 0.06 mg/kg/day (SED). These data suggest that a-damascone has no risk to human when it is exposed unintentionally to 0.01% of a-damascone in a set of cosmetic products, confirming its safety.

Keywords: a-Damascone, risk assessment, NOEL, SED, cosmetic

LBA21 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Risk assessment of glyoxal in cosmetic products

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Glyoxal is an organic compound with the chemical formula C2H2O2. It is the smallest dialdehyde and a yellow-colored liquid that evaporates to give a green-colored gas. Glyoxal is used as starting point for the production of a number of other compounds. Glyoxal has an ability to form heterocyclic compounds in the production of resins and for cross-linking functionalized macromolecules such as cellulose, polyacrylamides, polyvinyl alcohol, keratin and other polycondensates. Currently, the concentration limit of glyoxal is 0.01% in the finished cosmetic products. We performed risk assessment for glyoxal as a cosmetic ingredient. Systemic exposure dosage (SED) was estimated to be 0.03 mg/kg/day. And then no observed adverse effect level (NOAEL) was considered to 100 mg/kg/day. The margin of safety (MOS) for glyoxal in cosmetic products was calculated to be 3,333.33 based on 100 mg/kg/day (NOAEL) / 0.03 (SED). These data suggest that glyoxal has no risk to human when it is exposed to 0.01% of glyoxal in the finished cosmetic products, confirming its safety.

Keywords: Glyoxal, risk assessment, NOAEL, SED, cosmetics

LBA22 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Human risk assessment of resorcinol in cosmetic products

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Resorcinol is one of the main natural phenol extracts isolated from argan oil. Resorcinol is mainly used in oxidative hair colouring products, shampoos, hair lotions, peels and anti-acne creams. In Korea, the Ministry of Food and Drug Safety (MFDS) and in Japan, resorcinol have regulatory limit in use for maximum concentration of 0.1% in cosmetic products. In Europe, resorcinol when it functions as oxidizing colouring agent for hair dyeing, is limited up to 1.25% for cosmetics after mixing in a 1:1 ratio with hydrogen peroxide. And when resorcinol functions as hair lotion and shampoo, its limited use is 0.5%. Assuming maximum concentration of resorcinol (0.1%): the no observed adverse effect level(NOAEL) is estimated to be 80 mg/kg bw/day, when Spraque-Dawley rats were orally administered resorcinol for 13 weeks (93days). The dermal absorption of resorcinol is 0.76%, and systemic exposure dose (SED) is estimated to be 0.00228 mg/kg bw/day. Consequently, the margin of safety (MOS), NOAEL/SED, is calculated to be 35,087.71. Taken into consideration of conservative estimation, resorcinol appears to exert no serious risk for human health and is considered safe as a cosmetic ingredient up to 0.1% in cosmetics.

Keywords: Resorcinol, Risk assessment, Cosmetic, Safety

LBA23 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Risk assessment of 3-Propylidene phthalide in cosmetic products

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3-Propylidene phthalide has been used in cosmetics as soap, detergent, creams, lotions, and perfume. Administrative standard of 3-propylidene phthalide used is less than 0.01% in Korea and Europe. Available toxicological data for 3-propylidene phthalide indicated that 3-propylidene phthalide is not carcinogenic in animals. The no observed adverse effect level (NOAEL) was estimated to be 5.42 mg/kg/day, when rats were orally administered 3-propylidene phthalide for 13 weeks. The concentration of the administered 3-propylidene phthalide in accordance with the period, was different (47ppm for 0-4 weeks, 78ppm for 5-10 weeks, 94 ppm for 11-13 weeks). A risk assessment was carried out in cosmetics by no observed adverse effect level (NOAEL) / systemic exposure dosage (SED). Thus, risk for 3-propylidene phthalide in cosmetic products was calculated to be 180.66 based on 5.42 mg/kg bw/day (NOAEL) / 0.03 mg/kg/day (SED). These data suggest that 3-propylidene phthalide has no harm to human when it is exposed unintentionally upto 0.01% of 3-propylidene phthalide in a set of cosmetic products, confirming its safety.

Keywords: 3-Propylidene phthalide, risk assessment, NOAEL, SED, cosmetic

LBA24 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Human risk assessment of lithium hydroxide in cosmetic products

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The inorganic hydroxides, calcium hydroxide, lithium hydroxide, magnesium hydroxide, potassium hydroxide, and sodium hydroxide, have been used as pH adjusters in cosmetic products. Among them, lithium hydroxide is commonly used as the ingredient for hair straightening agents. The concentration of lithium hydroxide is limited as less than 4.5% for hair straighteners in Korea regulated by the Ministry of Food and Drug Safety (MFDS). However, this substance was banned to be mixed with other products except hair straighteners. In European Union, the regulatory limit for lithium hydroxide concentration is less than 2% (general use), and less than 4.5% (professional use) in hair straighteners. Assumption of using maximum concentration of lithium hydroxide: the no observed adverse effect level(NOAEL) is estimated to be 4.13 mg/kg bw/day, when humans were orally administered lithium hydroxide for long period of time to treat bipolar disorder. Systemic exposure dose (SED) is estimated to be 0.3 mg/kg bw/day. Consequently, the margin of safety (MOS), NOAEL/SED, is calculated to be 13.77. Therefore, lithium hydroxide is safe for use in cosmetic products. (If the NOAEL value is based on human test data, it is considered safe in case of MOS value more than 10).

Keywords: Lithium hydroxide, hair straighteners, Risk assessment, Cosmetic, Safety

LBA25 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Risk assessment of calcium hydroxide in cosmetic products

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Calcium hydroxide has been used in cosmetics as hair straighteners and pH adjusters. The use of calcium hydroxide may not exceed 7% in hair straighteners containing calcium hydroxide and a guanidine salt, must have a pH below 12.7 when used as a pH adjuster in depilatories, and must have a pH below 11 in all other uses. Calcium hydroxide has the highest reported maximum concentration of use; up to 13.2% in rinse-off shaving preparations. However, it is only used up to 0.5% in leave-on products (deodorants). The chronic exposures caused local hyperplasia, metaplasia and inflammation but there is no indication of a potential carcinogenic effect of calcium hydroxide due to their similar or lower pH. The no observed adverse effect level (NOAEL) was estimated to be 1200 mg/kg/day, when rats were orally administered calcium hydroxide group 1; for 6 weeks before mating and group 2; for 20 days during mating period, pregnancy. Based on this information, systemic exposure dose (SED) and margin of safety (MOS) for hair straightener were calculated to be 4.084 mg/kg/day and 293.82, respectively. The MOS values indicate that calcium hydroxide is considered safe as a hair straightener.

Keywords: Calcium hydroxide, risk assessment, NOAEL, SED, cosmetic

LBA26 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Risk assessment of silver nitrate in cosmetic products

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Silver nitrate (AgNO3) is one of the most abundant silver compounds in the environment. The main indication for the use of nano - silver is its antibacterial or biocidal activity, which is why silver compounds have been used for centuries in health care products as an antiseptics. In Korea and Europe, the use of silver nitrate (AgNO3) may not exceed 4% in eyelashes and eyebrow coloring products. Exposure to soluble silver compounds may produce other toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory and intestinal tract, and changes in blood cells. Silver and nano - silver have been clearly shown to have toxic potential, although general toxicities seem to be low in humans. The no observed adverse effect level (NOAEL) was estimated to be 150 mg/kg/day, when rats were orally administered 1.5, 15, 150 mg/kg bw/day silver nitrate for 30 days. Based on this information, systemic exposure dose (SED) and margin of safety (MOS) were calculated to be 1.6667x10-4 mg/kg/day and 899,982, respectively. The MOS values indicate that silver nitrate is considered safe as an eyelashes and eyebrow coloring products in humans.

Keywords: Calcium hydroxide, risk assessment, NOAEL, SED, cosmetic

LBA27 [Accepted:Poster Presentation] [Nanomaterials]

Lung exposure to multiwalled carbon nanotubes alters estrous cyclicity in mice

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Carbon nanotubes attract huge industrial interest due to their unique properties. Multiwalled carbon nanotubes (MWCNT) consist of concentric graphene tubes with diameters of up to 100 nm. Exposure is anticipated to be of concern in occupational settings primarily by inhalation. Airway exposure to nanoparticles causes lung inflammation in experimental studies. As experimental animal studies show that inflammation may interfere with the female hormonal reproductive axis, we hypothesized that lung exposure to MWCNTs could potentially interfere with female fertility and studied this in mice. Mature female C57BL/6J mice were intratracheally instilled once with 67μg of MWCNTs and females underwent daily vaginal lavage for 14 days pre- and 14 days post-exposure. Microscopic examination hereof served as basis for estimation of estrous cycle length. Two weeks after exposure, lung inflammation was assessed by differential cell count of bronchoalveolar lavage fluid. Lung inflammation was evident in exposed females, as judged by the differential cell counts. Mixed model analysis of cycle lengths indicated differential and highly statistical significant effects of the exposure. Exposure prolonged the length of estrous cycle in which exposure took place by two days compared to the pre-exposure cycle. The subsequent cycle was however significantly shortened. Both exposure and post-exposure values varied statistically significantly from cycle lengths in control females. The changes observed in estrous cyclicity are concordant with findings in studies of inflammation and female reproduction. Exposure to MWCNTs could potentially interfere with the female reproductive cycle, and thereby affect the ability to achieve pregnancy.

Keywords: Carbon nanotubes, reproductive toxicology, estrous cycle, female fertility

LBA28 [Accepted:Poster Presentation] [Juvenile Toxicology]

Oral Study on Toxicity and Toxicokinetics in Juvenile Rats

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Wuxi apptecc

The test article (TA0529) currently is being explored as anti-virus candidate not only in adults but only in pediatrics. The study is designed to evaluate the potential toxicity profile of TA0529 when administered via oral gavage to juvenile rats once daily at the dosages of 50, 150, and 450 mg/kg/day for 28 days, from PND4 (postnatal day 4, the day of birth is considered as PND0) to PND31. After single oral dosing, exposure level in juvenile rats was slightly higher than adult rats. Cmax on PND4 was up to 1.7-fold higher and AUC0-24h on PND 4 was up to 3.4-fold higher than adults at 8 weeks of age. After repeated dosing for 28 days, exposure represented by Cmax and AUC0-24h was similar in juvenile rats on PND 35 and adult animals at 12 weeks of age. Treatment related clinical observations including coat soiled with yellow materials principally at the anogenital region were observed at 450 mg/kg/day. Slight decreases of body weights (up to 11.1%) were observed in males and females during lactation and post lacatation at 450 mg/kg/day. Treatment related delayed effect on respiratory parameters (increased tidal volume and derived minute volume) was observed at 450 mg/kg/day. Slight increases in liver, spleen and thyroid weight correlating with minimal microscopic changes in these organs were observed at 450 mg/kg/day. All were considered non-adverse. In conclusion, the no observed adverse effect-level (NOAEL) of TA was considered to be 450 mg/kg/day in juvenile rats.

Keywords: Juvenille, Toxicity, Toxicokientics

LBA29 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Risk assessment of Acetyl Hexamethyl Tetralin (AHTN) in cosmetic products

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AHTN (Acetyl Hexamethyl Tetralin) is a member of a group of substances used in fragrances and known as the polycyclic musk.
It applied in a fragrance mixture used in a wide variety of consumer products such as perfumes, cosmetics, household and air fresheners. A few studies performed have shown that AHTN exhibits slight erythema in rabbit. However, AHTN has no evidence of carcinogenic and mutagenic properties. AHTN has been restricted to be used at concentrations of 0.1% in leave-on product, 1% in hydro alcoholic products, 2.5% in fine fragrance, 0.5% in fragrance cream and 0.2% in rinse-off products by EU(exception of oral use) and South Korea. A risk assessment for AHTN was carried out as a cosmetic ingredient. Using SED (systemic exposure dose) calculated to be 0.015141 and NOAEL(no observed adverse effect level) of 5mg/kg/day, a margin of safety (MOS=NOAEL/SED) is estimated to be 330.22. It is far greater than 100, a critical value of risk determination for human safety criteria. A review on safety evaluation and risk assessment of AHTN demonstrates that AHTN is considered safe as a cosmetic ingredient.

Keywords: Acetyl Hexamethyl Tetralin(AHTN), risk assessment, NOAEL, SED, cosmetic

LBA30 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Quinine: Safety evaluation & Risk assessment in cosmetic

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Quinine belongs to the group of alkaloids and is used to treat malaria. Quinine has been widely used in cosmetics and food sector as a fragrance ingredient and conditioning agent. According to the toxicity data, quinine sulphate does not induce carcinogenicity. However, quinine may occur health disorder in the mother and child, when pregnant women consume any quinine-containing soft drinks. In EU (European Union) and South Korea, the restriction for quinine concentration is up to a maximum concentration of 0.2% in hair lotion and 0.5% in hair rinse-off product. Other products prohibit the combination of quinine and its salts. A risk assessment for quinine as a cosmetic ingredient followed SCCS (Scientific Committee on Consumer Safety) and MFDS (the Ministry of Food and Drug Safety) guidelines. Based on no observed adverse effect level (NOAEL) of 60mg/kg/day, which was obtained from 13-week repeated oral toxicity test, systemic exposure dose (SED) is estimated to be 0.0226mg/kg/day and the margin of safety (MOS=NOAEL/SED) is calculated to be 2,654.86. The MOS value indicates that exposure to quinine in the existing regulation is considered safe as a flavouring and conditioning agent in cosmetics.

Keywords: Quinine, risk assessment, NOAEL, SED, cosmetic

LBA31 [Accepted:Poster Presentation] [Alternative Animal Models]

Alternative nonclinical assessment of human fetal risk of recombinant VWF

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INTRODUCTION: Baxalta is marketing a human recombinant von Willebrand factor (rVWF) product to treat von Willebrand disease. Standard reproductive and developmental toxicity studies in rats and rabbits, as recommended by ICH, would not add information on the safety profile of rVWF, as antibody formation in animals after repeated dosing with human rVWF is not relevant to the clinical setting. We therefore sought an alternative to animal testing, in line with the principles of the 3Rs (Reduction, Refinement, Replacement).
OBJECTIVE: To assess the human fetal risk of rVWF using i) literature and available nonclinical data, and results from ii) an in vivo placental transfer study in rabbits and iii) an ex vivo human placental transfer study.
METHODS: The rabbit study was conducted according to standard protocol. In the ex vivo study, placentas obtained up to 30 minutes after delivery from normal human pregnancies were actively perfused with physiological and up to 10-fold concentrations of rVWF (n=5/group) for 120 minutes and compared with positive controls (antipyrine, n=5). Perfusate samples from the maternal and fetal circuit were collected every 30 minutes for detection of rVWF.
RESULTS: No placental transfer of rVWF was detected in rabbits. In the ex vivo study, as expected, rVWF was only detected on the maternal side, demonstrating that rVWF does not pass the human placenta.
CONCLUSIONS: The results of the in vivo rabbit and ex vivo human placental transfer studies indicate that the risk of direct harmful effects of rVWF on the fetus is low.

Keywords: von Willebrand disease, recombinant von Willebrand factor, safety, placental transfer

LBA32 [Accepted:Poster Presentation] [Biomarkers]

Human biomonitoring of multiple mycotoxins in Golestan Province, Northeastern Iran with GC-MS/MS

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Mycotoxins are naturally occurring secondary metabolites of fungi commonly found to contaminate large volumes of staple food. Human exposure to these natural toxins is predominantly through consumption of contaminated foods. Golestan province, located in northeastern Iran, has been known as a high risk area for esophageal cancer (EC). In order to understand the possible links between mycotoxins and human disease, the relationships between multimycotoxins contamination of EC patients and healthy close relative them urine samples is investigated. The early morning first urine Sample was carried out from esophageal patients as case group (n=17) that confirmed disease with pathological procedure and healthy close relative of them as a control group (n=10). Multi-mycotoxin determined for (DON, 3ADON, 15ADON, FUSX, NIV, NEO, HT2, T2, ZAN and ZON) after extraction and analysis with GC-MS/MS method and reported after adjusted with urine creatinine (µg/g) (Rodríguez-Carrasco et al. 2014). Urine mycotxin positive sample in case and control group were 17.6%, 40% respectively. DON was only detected in one of the control group with 3.26 µg/g creatinin. NEO was found in two case and three control group (Mean 10.95, 6.0 µg/g crea respectively). T2 detected from one of the case group (Mean 435.78 µg/g).HT2 was found in three cases and one of control group (Mean 1511.43, 7.87 µg/g respectively). Exposure to mycotoxins in this province is very different pattern in comparison with other study. Patient's (EC) have a control diets and treatment with drugs may be influence to detection mycotoxins.

Keywords: Urine,Zearalenone, Mycotoxin,Biomarker, GC

LBA33 [Accepted:Poster Presentation] [Food Safety / Food Allergies]

A novel method for multi-mycotoxins detection in chicken liver using Gas Chromatography coupled with Triple-Quadrupole Mass Spectrometry (GC-QqQ-MS/MS)

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Mycotoxins are toxic metabolites elaborated by several fungi and constitute an important group of contaminants with diverse potent toxic effects in humans and animals. Presently, more than 450 secondary metabolites are identified and characterized, however around 30 are of real concern and represent a serious threat for human and animal health all over the world such as carcinogenicity, genotoxicity, teratogenicity, neurotoxicity, hepatotoxicity, nephrotoxicity and immunosuppression. The main objective of the study was to develop a reliable and sensitive method for the analysis of ten mycotoxins including patulin (PAT), zearalenone (ZON) and eight trichothecenes (NIV, FUS-X, DAS, 15-ADON, NEO, DON, T-2 and HT-2) in chicken liver. Homogenized liver samples were extracted with acetonitrile followed by centrifugation, and cleaned-up by dispersive solid phase extraction (d-SPE) before being injected into gas chromatography tandem mass spectrometry (GC-MS/MS). The method was successfully validated according to the European guidelines recommendations with satisfactory recoveries (54–109%), and good linearity between limits of quantitation (LOQ) and 100 times LOQ. Limits of detection (LOD) and LOQ were ≤ 1 and ≤ 10 ng/g, respectively. The validated method was applied for the analysis of 50 liver samples. The mean concentration of mycotoxins found was ranged from 1.60 to 22.96 µg/kg in the analyzed samples. Additionally, seventeen liver samples showed multi-contamination by at least 2 up to 5 different mycotoxins, being DON, NIV, NEO and FUS-X, the most frequent combination found. The results indicated that the present method could be successfully applied for mycotoxins analysis in liver and other tissue samples.

Keywords: Chicken liver, Gas chromatography, Mass spectrometry, Method validation, Trichothecenes.

LBA34 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Early biological effects in children exposed to different levels of PM0.5 in Perugia (Italy)

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Exposure day-to-day particulate matter (PM) air pollution is correlated to increased risk of various adverse health outcomes. In particular, the children are more vulnerable than adults to the effects of airborne agents because their lungs are still developing, they spend more time outdoors, and they breathe faster than adults do. Our work was part of the MAPEC (Monitoring Air Pollution Effects on Children for supporting public health policy) project, a multicentric cohort study that involves 1,000 primary school children (6-8 years old) in 5 Italian cities (Brescia, Lecce, Perugia, Pisa and Turin), with different degrees of pollution. The aim of this study was to identify markers of early biological damage, such as micronuclei (MN), predictive of chronic diseases onset in older adulthood. Child exposure to urban air pollution was evaluated by collecting PM0.5 samples in four schools of Perugia, on the same day of biological sampling. The micronucleus cytome assay was performed in exfoliated buccal mucosa (BM) cells of children. The cells, collected by brushing the inside of the cheek with a toothbrush, were fixed on microscope slides and stained with Feulgen/LightGreen for both bright field and fluorescence microscope analysis. Ambiental and biological sampling were repeated in winter 2014 and in spring 2015. The results showed a decrease in MN frequency in spring compared to winter season, according to the monitored level of air pollution

Keywords: air pollution, PM0.5, children, buccal cytome assay

LBA35 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Chemical analysis, mutagenicity and genotoxicity of PM0.5 collected in Perugia (Italy)

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Particulate matter (PM) air pollution has long been recognized as a threat to human health, causing both short and long term adverse effects. Several components of ultra-fine PM, such as polycyclic aromatic hydrocarbons (PAHs) and nitrated polycyclic aromatic hydrocarbons (nitro-PAHs), have been identified as being of greatest concern for their toxic effects in humans. The aim of this work, which is part of the MAPEC_LIFE (Monitoring Air Pollution Effects on Children for supporting public health policy) study, was to evaluate the toxicity of PAHs and nitro-PAHs in ultra-fine air particulates (PM0.5) collected in four different areas in Perugia (Italy), during two different seasons (winter 2014 and spring 2015). PM0.5 was collected on glass fibre membranes using a high-volume air sampler. After 72 hours, the membranes were subjected to chemical analysis to measure the concentration of PAHs and nitro-PAHs. PM0.5 organic extracts were analysed for their ability to cause mutagenicity on four different *S. typhimurium strains* (Ames test) and genotoxicity in a lung adenocarcinoma (A549) cell line (comet assay and micronucleus test). Chemical analysis showed that PM0.5, PAHs and nitro-PAHs levels were higher in winter samples than in the spring ones. PM0.5 organic extracts showed mutagenic effects only in the YG1024 strain, with an increased activity when the S9 fraction was used, thus indicating the presence of promutagenic chemicals. No genotoxicity was observed in A549 cells following exposure to PM0.5 extracts collected in winter and spring, as assessed by the comet assay and the micronucleus test.

Keywords: PM0.5, toxicity, Ames test, comet test, micronucleus assay

LBA36 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

Genotoxicity and Cytotoxicity Profile of E-Cigarettes: An Interdisciplinary Review

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Electronic Cigarettes also named as e-cigarettes have been intensively used in the World as increasing concern with their toxicity and safety. Little is known regarding to effects on e-cigarette users and second-hand smokers as well. Concerns mostly involve the toxicity of highly concentrated nicotine and intentional misuse. Current findings indicated that e-cigarette liquids seems to be any toxicity in vitro systems.However, it may cause adverse respiratory outcomes in long term and further research needs to be carried out. This review attempts to make a summary of recent research on e-cigarettes, including their toxicity and health outcomes.

Keywords: e-Cigarettes, toxicity,genetics,epigenetics

LBA37 [Accepted:Poster Presentation] [Carcinogenesis]

Pendimetalin carcinogenic potential identification by means of thyroid function study

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Well known that original pendimetalin is carcinogen for laboratory animals (Group C by US EPA) with hormonal mechanism of action.
AIM: to determine carcinogenic potential of generic pendimethalin by identifying violations of thyroid function.
The experimental study was performed in accordance with the Endocrine Disruptor Screening Program (OCSPP Guideline 890.1500) on 60 Wistar Han rat (15 males per group). Pendimetalin was administered per os daily at doses 5, 10, 500 mg/kg from 23 to 53 postnatal days.
It was shown that pendimetalin at dose 500 mg/kg produced significant animal growth retardation and affected male pubertal development by criteria "age preputial separation". Treated animals showed increased kidney, liver weight, significantly decreased testis, epididymis, prostate, seminal vesicle, levator ani/bulbocavernosus muscle weight. Histomorphological evaluation of the thyroid revealed increased follicular cell height, reduced colloid. In the epididymis sperm was reduced. In the testes seminiferous epithelium atrophy and Leydig cell hyperplasia was found.
It is concluded that the studied pendimetalin at dose 500 mg/kg produces signs of increased thyroid morphofunctional activity and hormones imbalance.

Keywords: Pendimetalin, thyroid, cancer, hormones

LBA38 [Accepted:Poster Presentation] [Human and Environmental Risk Assessment]

E-Cigarette Smoking and the Risk of Cancer: A Systematic Review

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E-cigarettes, most of which consist of a battery, a cartridge, and a vaporiser, contain a liquid mixture inside their cartridges which is heated and vaporised by the atomizer. According to the Centre for Disease Control and Prevention (CDC) in America, 3.7% of American adults (more than 9 million individuals) regularly use e-cigarettes. Currently, no research has clearly shown the effect of e-cigarettes on the prevalence of cancer.However,some studies have shown that carcinogenic substances may be found inside e-cigarette liquids. In this review, we examined the recent research on the effects of e-cigarette usage with carcinogenesis, regarding to chemicals in e-cigarette liquids.

Keywords: E-cigarette,cancer, cancer risk,carcinogens

LBA39 [Accepted:Poster Presentation] [Clinical Toxicology]

Methemoglobinemia: Dapsone chronic poisoning

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Methemoglobinemia is a blood disorder, which can be caused among other causes by drugs such as dapsone (rare). We report a case.
A 50 years old male without known drug allergic reactions had a dermatitis herpetiformis that was being treated with dapsone 100 mg daily orally for 32 years without any analitical control. He went to the hospital with flu symptoms and confusion, asthenia, cyanosis of perioral mucous surface with severe hypoxemia. In the emergency department, he presented pancytopenia (hemoglobin 5,5 g/dl, hematocrit 15,5 %, red blood cells 1,56 mill/mm³, leukocytes 1 mil/mm³ and platelets 73 mil/mm³). Dapsone therapy was discontinued. Two days later, chronic poisoning was confirmed by arterial blood gas analysis: total hemoglobin 5,1 g/dl, oxyhemoglobin 83,6 %, carboxyhemoglobin 3,9 %, methemoglobin 6,2 % and deoxyhemoglobin 6,3 %. For seven days he was treated with 1 g intravenous ascorbic acid and all analytical values improved (total hemoglobin 8 to 9,7 g/dl, oxyhemoglobin 87 to 91,2 %, carboxyhemoglobin 0 to 2,9 %, methemoglobin 7,6 to 1,7 % and deoxyhemoglobin 5,4 to 4,2 %, hematocrit 15,5 to 29,3 %, hemoglobin 5,5 to 9,3 g/dl, red blood cells 1,56 to 2,68 mill/mm³, leukocytes 1 to 2,1 mil/mm³ and platelets 73 to 98 mil/mm³). A month later, he was revised, being analytic levels normalised.
We have observed that analytical values and symptoms control is necessary by physician during chronic treatment with dapsone to avoid or minimize adverse events. The ascorbic acid treatment has shown to be effective against dapsone methemoglobinemia.

Keywords: Dapsone, methemoglobinemia, ascorbic

LBA41 [Accepted:Poster Presentation] [General Toxicology]

7-hydroxycoumarin and its mixture with ethanol toxicity profile. Nonclinical study.

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7-hydroxycoumarin (7-HOC) is proposed to be used as a low-alcohol beverages fluorescent dye. The safety/toxicity profile of 7-HOC and the toxicodynamic of its mixture with ethanol after acute and in course of repeat (28 days) doses were studied in laboratory animals. Various in-life, physiological, hematological, biochemical parameters, macro- and microscopic changes were examined. It was found that 7-HOC is low-toxic (DL>10000 mg/kg), not skin irritant, mild eye irritant, displays neither sensitizing, nor immunotoxic potential, produces weakly expressed functional cumulation. 7-HOC acute intoxication (5000 mg/kg) causes in rats transitional glycosuria associated with lowered blood glucose levels, decreased urea clearance. 7-HOC given orally (0, 20, 50, 200, 500 mg/kg) 3 month to rats of both sexes caused no death, body weight gain decrease (males only), mild behavioral changes, transitional anemia. Liver, kidney, small intestine were defined as target toxicity organs. It was set up subchronic toxicity NOEL for rats (20 mg/kg) based on carbohydrates and lipids metabolism disturbance (the blood glucose level decrease, the serum triglyceride level rise) accompanied by mild lipid accumulation in hepatocytes, small intestine mucosa dystrophic changes. Mode of acute and subacute action of 7-HOC and ethanol mixture given orally was identified as mainly independent, antagonism by 7-HOC hypoglycemic effect were found for mixture of 7-HOC (200 mg/kg) and ethanol (750 mg/kg) in 28 day repeat dose study in rats.

Keywords: 7-hydroxycoumarin, ethanol, acute, repeat dose, mixture toxicity

LBA42 [Accepted:Poster Presentation] [Biomarkers]

The effects of thiamethoxam and lambda cyhalothrin, individually or in combination, on biomarkers of oxidative stress, neurotoxicity and endocrin disruption in the liver of Oreochromis niloticus

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This study aimed to determine the impacts of the neonicotinoid insecticide thiamethoxam and pyrethroid pesticide lambda cyhalothrin on the liver of Oreochromis niloticus. The fish were sampled after a 15-days exposure to thiamethoxam and lambda cyhalothrin, individually or in combination, followed by a 7-days recovery period to measure endocrine disruption, neurotoxicity, and oxidative stress. The exposure resulted in alterations in estrogen and testosteron levels. A marked decrease in the activity of acetylcholinesterase was also observed. Thiamethoxam and lambda cyhalothrin, individually or in combination, exposures induced oxidative stress as reflected by the significant increase in lipid peroxidation and glutathione contents, hepatic 7-ethoxyresorufin O-deethylase, superoxide dismutase and catalase activities, which initiates cellular defense against oxidative stress, with significant decrease in glutathione S-transferase activity. The results showed the potential neurotoxicity of pesticide exposures in the fish. Therefore, the present study shows that O. niloticus developed adaptive responses to overcome the oxidative stress following pesticide exposures, but fish were unable to overcome the stress of pesticides. There also exist low testosteron and estradiol levels in exposed fish, which is attributed to the capability of pesticides to impair steroid hormone levels. Thiamethoxam and lambda cyhalothrin have an oxidative-mediated endocrine disruption effects. These results suggest that selected biochemical responses in the liver of O.niloticus could be used as potential biomarkers for risk assessment of aquatic environment.

Keywords: Pesticides,fish, endocrine disruption, neurotoxicity,enzymes

LBA43 [Accepted:Poster Presentation] [Nanomaterials]

Toxicity assessment of silver nanoparticles complexed with humic acid using zebrafish (*Danio rerio*) as a model

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Silver nanoparticles (AgNPs) are being introduced into the consumer market significantly and can enter in aquatic environments forming sediments by adsorption with humic acid (HA). In this work, toxicological effects of AgNPs in the presence of HA were assessed in adult zebrafish. Interaction between AgNPs and HA was characterized by TEM, FTIR-ATR, XPS. Fish (n=7/group) were exposed during 96h to 0, 10, 20, 30, 40 and 60 mg/L AgNPs, with and without the presence of 20 mg/L HA (AgNPs+HA and AgNPs, respectively). Organisms were euthanized to collect peripheral blood for comet assay, micronucleus-MNs and nuclear abnormalities-NAs tests. Gills, liver and intestine were collected for histopathological assessment and EDX was used to detect AgNPs in these samples. Although XPS indicated that AgNPs surface was not oxidized, FTIR-ATR and TEM analysis showed the interaction between HA and AgNPs. LC50 values were 25.0 mg/L (AgNPs) and 40.56 mg/L (AgNPs+HA). DNA fragmentation, MNs and NAs were not significant ($p>0.05$) for AgNPs+HA and AgNPs. Chloride cell and lamellar epithelial cell hypertrophy, lamellar epithelial lifting, mucosal cell hyperplasia, lamellar fusion and aneurysms were observed in gills. Histological changes were not found in intestine and liver, but silver was detected in the intestinal lumen by EDX, for AgNPs and AgNPs+HA. Despite, the biomarkers showed no significant differences between the AgNPs and AgNPs+HA groups, the decrease in the LC50 could be associated with the presence of HA, being able to influence the AgNPs behavior/bioavailability in aquatic environments.

Keywords: Zebrafish, Silver Nanoparticles, Humic Acid, Nanotoxicology, Aquatic Environments

LBA44 [Accepted:Poster Presentation] [Biomarkers]

Is 14-3-3 eta protein a useful biomarker for predicting the response to biologic therapy in patients with rheumatoid arthritis ?

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14-3-3 eta protein is a new biomarker used for the early diagnosis of rheumatoid arthritis (RA). We monitored the evolution of this protein under biologic DMARDs (bDMARDs) and we tested the possible predictive role of this biomarker on a group of patients treated with anti-TNF α agents. We have also assessed the status of this biomarker and response to anti-TNF α therapy..
It was a prospective and observational study including 64 patients followed 12 months with active RA that required treatment with bDMARDs.
mean age was 57.55 ± 9.427 years, of the 64 patients included in the study 59 (92.2%) were women and 5 (7.9%) men.
Following evolution of 14-3-3 eta protein regarding mean values (0.43± 0.591 ng/ml initial, 0.32± 0.452 ng/ml at 6 months and 0.28± 0.499 ng/ml at 12 months) there were no significant differences between evaluations (p=0.33079).
Regarding the predictive role to bDMARDs treatment, following baseline titres and EULAR response at 6 months, we observed significant differences between groups (p=0.0452). Nonresponders had higher mean values (0.99±0.888 ng/ml) than those with moderate response (0.28±0.469 ng/ml) and good response (0.51±0.580 ng/ml).
After 12 months of treatment, there were no significant differences between groups (p=0.3761)
Regarding pretreatment status for 14-3-3 eta protein and EULAR response at 6 and 12 months there were no significant differences.
Conclusion is that 4-3-3 eta could be one of the biomarkers for identifying pretreatment responder versus nonresponder patients.We observed reduction of 14-3-3 eta protein titers after starting anti-TNF treatment but there were not significant

Keywords: Rheumatoid arthritis, biologic therapy, predictors of response, biomarkers, 14-3-3 eta protein