plasma of the studies with PNS toxicity. None of the biomarkers were consistently changed in puromycin study presenting glomerular injury. In summary, NF-L is an emerging sensitive and specific biomarker in rat for detecting compound-induced central and peripheral NS injuries. While NF-L measurement alone cannot inform the original site of the injury, addition of biomarkers like Tau and NSE and analysis in both plasma and CSF can provide additional information of the origin of NS injury. These results demonstrate the utility of NS safety biomarkers in rat and provide additional supporting evidence for translating these biomarkers for use in clinical settings to further ensure patient safety.

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P02-07
Integrated approach to evaluate (immuno)toxicity of BDE-47 in female Balb-c mice

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Polybrominated diphenyl ethers (PBDE) are persistent additive flame retardants present in several consumer products; PDBE bioaccumulate and bio-magnify in the food chain being detected in several environmental compartments, including human matrices [1]. PBDE are known endocrine disrupters (ED) interfering with the thyroid hormone metabolism and inducing neurobehavioral, reproductive, developmental effects [2]. PBDE-induced immunotoxicity ranges from increased susceptibility to infections to the release of pro-inflammatory cytokines [3]. Among the congeners considered by European Food Safety Authority as of primary interest, BDE-47 is selected to investigate by an integrated approach the toxicological effects and the impact on immune response by the T-dependent antibody response assay.

Methods: Female Balb-c mice are treated per os by gavage with 0 (control, 200 microl of corn oil), 7.5, 15 or 30 mg/kg body weight of BDE-47 for 28 days and immunized with a single injection of keyhole limpet hemocyanin (KLH). KLH-specific IgM and IgG serum antibody are detected by ELISA. Histopathological analysis is performed on liver, spleen, pancreas and jejunum; gene expression profile of selected miRNA is performed on liver, namely: miR-155 regulating both adaptive and innate immune responses, miR-223 regulating cholesterol homeostasis, let-7a acting as tumor suppressor by repressing growth-signalling proteins.

Results: No changes in body weight gain and feed consumption or clinical signs of toxicity are recorded. Relative liver and pancreas weight is significantly higher in mice at the medium and high doses levels.

High levels of specific IgM, IgG1 and IgG2a are present in sera of mice four weeks after immunization with KLH. Exposure to medium and high dose of BDE-47 induced a significant decrease in the specific IgM and IgG1 response and a slight but not significant reduction of IgG2a production.

Histopathological analysis showed significantly increased diffuse and macrovesicular fatty change and glycogen accumulation in liver, atrophic villi with vacuolated enterocytes in duodenum and red pulp hyperplasia with macrophages and/or foamy macrophage in the spleen at the high dose of BDE-47.

The gene expression analysis showed that mir-155 is significantly induced at all BDE-47 doses, compared to mice treated with only KLH. Let-7a is significantly up-regulated by BDE-47 at the highest dose, in presence of KLH, compared to both mice treated with KLH alone or not treated; let-7a is up-regulated at the BDE-47 low dose compared to control mice. No significant modulation was observed for mir-223, although a borderline significance ($p = 0.0662$) in mice treated with KLH and BDE-47 at the low dose was noted compared to mice treated with KLH only.The preliminary results showed that BDE-47 can modulate immune response and miR-155 expression, and it can induce metabolic alterations in liver and pancreas, although apparently with a mechanism independent by mir-223 expression.

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P02-08
The influence of cryopreservation on yH2AX levels

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H2AX histone phosphorylation (yH2AX) occurs as a response to DNA double-strand breaks (DSBs), being increasingly used in human biomonitoring studies (HBM) in recent years, as a reliable biomarker of genotoxicity (DNA damage). HBM studies often involve the collection of a high number of biological samples, making necessary sample cryopreservation for later analysis. Nevertheless, information on the possible effects of cryopreservation in yH2AX levels, or on how samples should be handled after freezing is still limited.

For this purpose, in this study, yH2AX levels were determined by flow cytometry in peripheral blood mononuclear cells (PBMCs), obtained from 5 healthy adults. To detect the effect of cryopreservation, yH2AX levels from untreated and treated (bleomycin; 1 and 20 mg/mL; 4 h) samples were assessed before and after cryopreservation (−80°C; cells in FBS with 10% DMSO). Levels of yH2AX were determined in cryopreserved samples 1) immediately after thawing; 2) after overnight recovery in PBMCs cell culture media; and 3) after a 24 h stimulation period with phytohemagglutinin (PHA) 1%.

Data obtained show that the freezing process (−80°C) causes an increment in yH2AX levels, that can to some extent be reverted with PHA stimulation or overnight recovery in cells with high levels of damage (treated samples). In opposition, stimulation or overnight recovery is found to increase levels of yH2AX in samples with baseline levels (untreated samples), being preferable to analyze samples immediately after thawing. These results provide evidence-based data that may contribute to the future adoption of better sample