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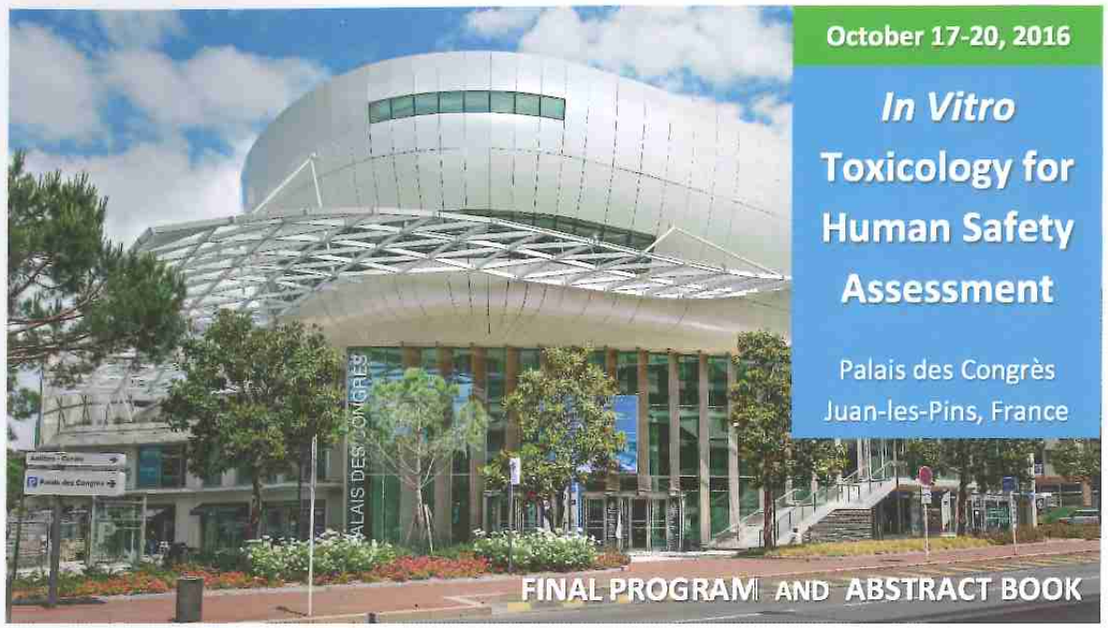

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Société Française de Toxicologie

ESTIV

Société de Pharmaco-Toxicologie Cellulaire

#	Session	Title	Authors	Affiliation
40	Extrapolation dose / modelling	Critical Membrane Concentrations of Amines in In Vitro and In Vivo Fish Bioassays	**Nynke I. Kramer**, Steven T.J. Droge, Joop L.M. Hermens	Institute for Risk Assessment Sciences, Utrecht University, the Netherlands

Neutral organic contaminants are assumed to be nonspecifically toxic at the baseline critical membrane burden (CMB) of ~100 mmol/kg lipid. Assuming a contaminant's octanol-water partition coefficient (logP) is equal to its membrane affinity (logKmw), the specificity of the toxic mode of action (or 'excess toxicity') can be estimated, where CMB in mmol/kg lipid = median lethal concentration (LC50) in mmol/L x logKmw in L/kg lipids. This assumption needs to be refined for contaminants that are predominantly present in their ionic form in toxicity assays as the CMB has only been determined for a large set of neutral contaminants. This is an important data gap in environmental risk assessment because the majority of pharmaceuticals and a substantial fraction of industrial chemicals are highly ionizable. The aim of this study was two fold: 1) to define the CMB for strong bases (pKa>9) using existing in vivo fish acute toxicity data from the US EPA Fathead Minnow Database, and 2) to determine whether this definition could be replicated in an in vitro cytotoxicity assay with a fish gill cell line (RTgill-W1). Membrane affinities for a series of amines listed in the US EPA's Fathead Minnow Acute Toxicity Database were determined using immobilized artificial membrane chromatography (IAM-HPLC), which allows for a direct interpretation of the critical membrane burden (CMB) in acutely exposed fish for these cationic chemicals. Accordingly, the CMB of thirteen amines with an unsure mode of action (MoA) was found to be within a very narrow range of 7.6±5 mmol/kg lipid, more than an order of magnitude lower than the baseline CMB for neutral chemicals. For the neurotoxic amines, the CMB of amphetamine was similar to those of the "unsure MoA" amines, while strychnine and nicotine showed an "excess amine toxicity" of a factor of 10, with CMB's of 0.6 mmol/kg lipid. RTgill-W1 cells were exposed for 48h to the abovementioned amines and cytotoxicity was assessed using the Alamar Blue assay. Test media were sampled before and after exposure and amine concentrations were analytically measured to define actual exposure concentrations. Unlike for neutral organic chemicals, in vitro median effect concentrations (EC50) for the tested amines were at least twofold higher than fish acute LC50 values. This suggests that the specific mechanisms by which amines accumulate or cause toxicity in vivo are not replicated in vitro.

44	Mixtures	Assessment of the genotoxic potential of pyrrolizidine alkaloids using human cell lines and the γH2AX In Cell Western technique	**Geert Stoopen**, Marc Audebert, Ad Peijnenburg	RIKILT, Wageningen University and Research Centre, Wageningen, the Netherlands

Pyrrolizidine alkaloids (PAs) are toxic compounds which are produced by a large number plant species and can be present as contaminants in food and animal feed. The toxicity of PAs in relation to human health risks has been subject for discussion in various organisations and agencies, such as WHO and EFSA. Currently, risk assessment of PAs is mainly based on data obtained through animal studies and relatively simple non-mammalian in vitro assays. The main toxicities elicited by PAs in experimental animals are genotoxicity, carcinogenicity (liver, pancreas) and hepatotoxicity. Furthermore, it appears that PAs have to undergo biotransformation to exert their toxicity. In order to contribute to a better evaluation of the mutagenic risks of PAs for human, the present work aimed to study the possible genotoxicity of PAs in human-based in vitro models.

For that purpose, three human cell lines, HepG2, LS-174T and ACHN, with different metabolic capacity (LS174T>HepG2>ACHN), were exposed to increasing concentrations of 12 PAs, i.e. riddelliine, retrorsine, echimidine, monocrotaline, lasiocarpine, senkirkine, heliotrine, jacobine lycopsamine, acetyllycopsamine, erucifoline and seneciphylline. Upon 24 hour exposure, cells were subjected to the recently developed γH2AX In Cell Western assay which is based on the quantification of phosphorylation of histone H2AX (γH2AX), a known sensitive biomarker of genotoxicity.

In LS174T cells, 9 out of 12 PAs induced phosphorylation of H2AX in the order heliotrine > 7-acetyl-lycopsamine > jacobine > lycopsamine > erucifoline >retrorsine > echimidine > lasiocarpine > riddelliine. Monocrotaline, senkirkine and seneciphylline were negative in LS-174T as well as in HepG2 cells. Also echimidine, lasiocaprine and riddelliine did not induce γH2AX in HepG2 cells.

In ACHN cells, only heliotrine and 7-acetyl-lycopsamine were slightly positive. Taken together, the outcome of this study indicated that many PAs are genotoxic in human cells and that biotransformation is an important factor for their genotoxic potential.