Additional Invited Speakers

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Toxicologic pathology aspects of chemically induced cardiovascular toxicity, as seen in National Toxicology Program (NTP) studies

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Potential cardiovascular toxicity of environmental agents and pharmaceuticals poses a major concern to health and regulatory authorities. Epidemiological studies have associated cardiovascular and respiratory morbidity and mortality with particulate matter (PM) air pollution, particularly in susceptible humans with concurrent cardiovascular and pulmonary diseases. Between 1961 and 1992, 131 drug products were withdrawn from the markets in Europe and the US. Ten of the 131 were withdrawn as the result of cardiovascular toxicity.

Studies in laboratory rodents are used to investigate the potential toxicity of various agents, identification and characterization of lesions suggesting cardiotoxicity are vital. Morphologic criteria have been described for degenerative myocardial lesions in rodents, but even with these criteria, differentiation of spontaneous from toxicity-induced lesions may be difficult. The histopathological pattern of lesion development may help determine whether the myocardial damage is due to injury of the coronary vasculature (in which case lesions tend to be multifocal) or due to direct myocardial cell toxicity (in which case lesions tend to affect much or all of the myocardium diffusely). In view of this observation, a retrospective light microscopic evaluation was performed on the hearts of F344 rats and B6C3F$_1$ mice from National Toxicology Program (NTP) studies on six chemicals that produced myocardial toxicity in order to provide a detailed morphologic characterization of spontaneous versus treatment-induced myocardial lesions (Jokinen et al., 2005). The findings at light microscopic evaluation, particularly when taken in conjunction with the results of other techniques, such as ultrastructural examination and special staining, may give a general indication of the potential mechanism of cardiotoxicity, and suggest possible areas for mechanistic studies to define more clearly the actual mechanism of toxicity. The lecture will present an overview of the morphologic aspects associated with chemically induced cardiovascular toxicity, as seen in the NTP studies.

Reference

Jokinen, Micheal P., et al., 2005. Cardiovasc. Toxicol. 5, 227–244.

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Preparation and validation of exposure and risk profiles for pesticide use in greenhouses

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Background: Greenhouses are peculiar indoor working environment, where mixtures of pesticides, variable according to crop and season, are used. Occupational exposure measurement is thus difficult and only seldom performed, thus making task-based semiquantitative estimates an attractive tool for exposure and health risk assessment. *Objective:* To provide a user-friendly, reliable description of risk through a minimum set of exposure indicators of work conditions and absorbed doses. *Methods:* (a) Indicators were chosen according to scientific and agronomic literature and task analysis. Different exposure conditions were ranked by attribution of scores (high, intermediate, and low) and a semiquantitative algorithm was implemented to model individual exposure. (b) Risk profile was applied to literature exposure studies to compare the agreement of model calculations to risk assessed by field measurements. (c) A targeted study was performed to evaluate the agreement of model estimates to measured exposure of greenhouse workers using chlorpyrifos-methyl. *Results:* Greenhouse workers resulted exposed to airborne concentrations below established safety standards; dermal exposure ranged from GM 20 ng/cm^2 outside clothing to GM 2 ng/cm^2 under clothing (range: 0.2–4100 ng/cm^2). Total Exposure Index was estimated: variables affecting pesticide exposure levels for each task were highlighted. These estimates are in line with published studies and with the measurements performed in the exposed workers. *Conclusions:* Exposure measurement, indirect assessment and derived risk profiles provide a reliable description of working conditions through a minimum set of indicators and with minimal use of expensive workplace or biological monitoring, thus qualifying as promising tools for pesticide risk evaluation in agricultural settings.

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Glutathione transferases and glutathionyl haemoglobin as biomarkers of oxidative stress in subjects exposed to low doses of 1,3-butadiene in a petrochemical plant

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Objective: Evaluate levels of glutathione transferases (GST) activity and glutathionyl haemoglobin (GS-SHb) in red blood cells following exposure to 1,3-butadiene (BD) in a petrochemical plant.

Methods: 42 BD-exposed and 43 no BD-exposed workers of a petrochemical plant, using 82 forest guards as controls were investigated measuring personal exposure to airborne BD, GST activity and GS-SHb in red blood cells, and GST T1, P1 and M1 genetic polymorphisms in peripheral blood lymphocytes.

Results: Median BD exposure was 1.5, 0.4, and 0.1 μg/m^3 in BD-exposed workers, no BD-exposed workers and forest guards.

Comparing petrochemical workers with forest guards we found a remarkable decrease of GST activity, and a significant increase of GS-SHb. Significant, but weak, positive correlations were found between BD exposure and GST activity, while a negative correlation was found between BD exposure and GS-SHb. Negative correlations between GST activity and GS-SHb were evidenced. No influence of age, cigarette smoking, alcohol intake on the investigated biomarkers was observed, while a reduced activity of GST in subjects bearing the T1-1 null genotype was found. Using multiple linear regression models up to 50.6% of the observed variability in GS-SHb and 41.9% of GST activity were explained by BD exposure, working setting, and GST T1 genotype.

Conclusion: These results suggest that working in a petrochemical plant induces an oxidative stress that impairs the GST balance in red blood cells, and that GS-SHb could be applied as biomarker of oxidative stress following chemical exposure.

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Tolerable drinking water limits for "non-relevant metabolites" of plant protection products: Testing strategies to derive health-based tolerable concentrations

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Limits for tolerable concentrations of "non-relevant metabolites" of plant protection products (PPPs) in drinking water between 0.1 and 10 μg/L are discussed depending on the toxicological information available. "Non-relevant metabolites" are degradation products of PPPs, which do not retain the targeted toxicities of PPPs. Analysis of health-based drinking water limits for registered PPPs with all necessary toxicology information available, result in tolerable drinking water concentrations (consumption of 2 L/day, body weight 70 kg, allocation of 10% of the TDI to drinking water) >10 μg/L for almost all PPPs forming "non-relevant metabolites". Since "non-relevant metabolites" are more polar and usually less toxic than PPPs, a limit of 10 μg/L is considered conservative. Before animal testing, all available information on the toxicity of the "non-relevant metabolites", the active PPP precursor, and structurally related compounds should be evaluated to develop structure-activity relationships. If these indicate concern or if the "non-relevant metabolite" is expected in drinking water at concentrations >3 μg/L, animal testing may be necessary. Two different testing strategies are proposed: (i) is relying on a 28-day oral toxicity study in rats (including endocrine-related endpoints) and a reproductive toxicity screen, (ii) uses an "enhanced" 90-day toxicity study (including endocrine endpoints) in rats to address both general toxicity and predict reproductive effects. For risk assessment of "non-relevant metabolites" a highly conservative margin-of-exposure approach is proposed with minimal MOEs of 3000, respectively 1000 when using the 28-day toxicity study or the 90-day toxicity study.

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Receptor-mediated toxicity: PPARα

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Peroxisome proliferators (PPs) are rodent nongenotoxic hepatocarcinogens that act via the PP-activated receptor α (PPARα) to cause peroxisome proliferation, induce DNA synthesis and suppress apoptosis in rodent hepatocytes. Extensive evidence suggests that the regulation of apoptosis and cell proliferation by PPARα is a key step in the carcinogenic mode of action, but it is unclear how PPARα mediates these cell growth changes. Since PPARα is a ligand-activated transcription factor, it seems reasonable to hypothesise that such cell growth regulation is transcription-mediated but as yet PPARα-regulated genes associated with cell growth have remained elusive. As well as a role for PPARα, evidence suggests that the growth response to PPARα ligands is dependent upon p38 MAP kinase. Interestingly, some transcription targets of PPARα appear dependent on p38-mediated phosphorylation of PPARα whereas others are not. These data suggest the potential for divergent downstream signalling from PPARα dependent upon survival signalling networks. In addition, there are marked species differences in response to PPARα ligands possibly mediated by both quantitative and qualitative differences in PPARα responses between rodents and humans. Recent data (Yang et al., 2008) have confirmed this by demonstrating differential regulation of several PPARα responsive genes in wild type versus PPARα humanised mice. Another area of current interest is the role of epigenetic modulation if the mode of action of PPARα. Specifically, recent data have demonstrated global and repeat sequence hypomethylation in response to PPs with significant implication for DNA stability (Pogribny et al., 2007). Overall, extensive evidence exists to demonstrate that PPARα-mediated rodent liver peroxisome proliferation and carcinogenesis but key steps in the mechanism remain to be elucidated.

References

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