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P21-60

Metabolic activities in Rainbow trout (Oncorhynchus mykiss) S9 fractions from liver and extrahepatic organs as an alternative *in vitro* ecotoxicity assessment approach

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Whole body biotransformation rate constants can be calculated using an appropriate *in vitro* to *in vivo* extrapolation (IVIVE) model. These models use CL,*In vitro*$_{,\,INT}$ rates derived with OECD Test Guideline 319B or 319A to estimate liver clearance rates, which are then extrapolated to a whole-body (*in vivo*) biotransformation rate constant. However, beside the liver, extrahepatic organs may also display Phase I and Phase II biotransformation activities and thereby play a role in metabolic clearance and bioaccumulation of compounds.

To adress these questions, we have maintained rainbow trout (Oncorhynchus mykiss) under controlled housing conditions according to OECD 319A/B. Specimens of eight sexually immature animals were harvested and pooled, including liver, gill, intestine, brain, heart and spleen. S9 fractions were prepared to determine the Phase I and Phase II enzyme activities by Liquid Chromatography-Mass Spectometry analysis. Cytochrome P450 activities, glucuronidation and sulfation activities were analyzed.

The liver displayed the highest Cytochrome P450 activities of all organs tested. Chorzoxazone Hydroxylase activity was only detectable in liver, 1-OH-Midazolam Hydroxylase activity was mainly restricted to liver, minor activities could be detected in intestine. However, Phenacetin-O-Deethylation was also detectable in other organs, with intestine, gill and spleen contributing 34, 18 and 11% of the total enzyme activity. Diclofenac-hydroxylase activity was present in all organs, as well as Bupropion-4-Hydroxylase activity, which was more or less evenly distributed among all organs. Phase II activities were detected in the liver, gill, intestine and heart, but not in spleen or brain.

In summary, the liver is the major organ for detoxification of compounds. However, extrahepatic organs, mainly intestine and gill, but also the brain, heart and spleen exhibit certain cytochrome P450 activities. Phase II enzyme activities were also detected in the intestine and gill. Our results suggest that extrahepatic organs, mainly intestine and gill, should also be taken into account when bioaccumulation and *in vitro* clearance rates are determined for IVIVE modeling in rainbow trout.

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P21-62

The copper-based nanopesticide Kocide 3000® worsens development of colitis in mice as a function of sex, and disrupts the intestinal barrier function in an enteroid-derived epithelial cell monolayer model from mouse gut organoids

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Nanoparticles (NPs) can cross gut barrier, interact with immune cells and alter gut microbiota with health concerns. Kocide 3000® (K3) is a copper ($Cu(OH_2)$)-based nanopesticide, but the intestinal effects of Cu-NPs compared to Cu-based conventional (non-nano) pesticide have not received attention. We aim to evaluate in mice the impact of K3 exposure on microbiota-dependent colitis, and the gut barrier tolerance to K3 using an enteroid-derived monolayer (EDM) model and immune cells from mesenteric lymph nodes (MLN) in comparison to Kocide 2000® (K2), a non-nanosized form.

Female mice were exposed to a control, K3 or K2-enriched diet from pregnancy to weaning of pups. All doses were adjusted for Cu content, and mice were exposed to 0, 0.25 and 25mg of K3/kg bw/d, or 0.215mg of K2/kg bw/d. Male and female offspring (F1) were fed as their mother until adulthood and colitis was induced by dextran sulfate sodium (DSS; 2% in drinking water) for 7 days, followed by a 5-day recovery, and disease activity was monitored daily. EDM were cultured from intestinal crypt stem cells of naïve mice and exposed for 24h to Cu-adjusted doses of K3 (0.025–25µg/ml) or K2 (0.0215–21.5µg/ml). LDH release was assessed for cytotoxicity, and gene expression of gut barrier homeostasis markers by qPCR. Genotoxicity was studied by 53BP1 and γH2AX immunostaining. MLN cells from naïve mice were stimulated (PMA/ionomycine or anti-CD3/anti-CD28) in the presence of K3 or K2 for 48h, and cytokine levels measured for direct effects of K3 and K2.

At 25mg of K3/kg/d (Cu NOAEL), F1 males were more susceptible to DSS-induced colitis, while females were protected. Colitis activity at 0.25mg of K3/kg/d and 0.215mg of K2/kg/d (Cu ADI) was not different from controls. To decipher the K3-related mechanisms in support of the colitis flare in males, the direct impact of K3 on EDM and MLN cells was evaluated. A slight cytotoxicity on EDM was noted at 25µg/mL of K3 only. Neither genotoxic nor oxidative stress effects were reported for K2 and K3 regardless of dose. Exposure of the EDM to K3 resulted in a decrease in the stem cell marker *Lgr5*, suggesting impaired epithelial renewal, while an increased mucin-producing gene *Muc2* expression occurred. Downregulation of the antimicrobial peptide genes *S100a8* and (dose-dependently) *Reg3*γ occurred after K3 exposure, possibly promoting gut dysbiosis. Interestingly, EDM alterations were not reported after K2 treatment, highlighting K3 effects linked to nanoformulation. Finally, no effect in cytokine production by MLN cells was reported whatever the treatmentThese data show that exposure to long-term K3 exposure through the diet aggravates colitis in a sex-dependent manner. As the immune cells do not respond to K3, the exacerbation of colitis could be due to Cu-NP-evoked disruption of the gut barrier integrity and its secretory functions. In addition to proper biocidal properties of Cu, this could aggravate the pro-inflammatory dysbiosis in DSS-treated males.

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P21-63

Towards an understanding of the relative toxicity of nanoparticles from different transport sources

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In recent decades the focus has increased on the smallest size fraction of particles found in urban air (less than 100 nm), called ultrafine particles or nanoparticles. A vast variety of health effects have been linked to particulate matter (PM) inhalation, including respiratory and cardiovascular diseases as well as cancer. There is, however, still a lack of understanding regarding to what extent nanoparticles from different sources differ in toxicity and health effects [1]. Within the project called nPETS (nanoparticle emissions from the transport sector: health and policy impacts), we have explored the toxicity of a range of nanoparticles formed in various transport systems. These include laboratory settings in which we generated nanoparticles from rail systems, as well as brake- and clutch wear. We also collected nanoparticles at different sites in Europe including in a road tunnel, subway, harbor, and airport. For road tunnel, subway and some brake materials, we compared the toxicity to larger sized particles (micron sized or PM2.5). We used the lung epithelial cell line A549 as well as differentiated THP-1 (dTHP-1) and explored cytotoxicity, DNA-damage (comet assay) and inflammation (secretion of IL-8, IL-6, TNFα and IL-1β). For some materials we mainly focused on inflammation in dTHP-1 cells.

The results showed that both nano- and micron sized particles from the road tunnel and subway caused DNA strand breaks and secretion of inflammatory cytokines. The cytokine secretion was mainly evident in the dTHP-1 cells and the micron-sized particles appeared more inflammatory. Brake wear particles showed in general low cytotoxicity and little inflammatory potential, except for one material (NAO, Non-Asbestos Organic) in nano-size. The harbor and airport nanoparticles (from Barcelona) showed low cytotoxicity but a high inflammatory potential. We are now exploring the possibility to generate "toxicity scores" of the nanoparticles from the different sources and to link the effects to their chemical composition.

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P21-64
Characterization of the toxic effects by the marine toxin ovatoxin-a on human skin keratinocytes

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Ovatoxin-a (OVTX-a) is the major palytoxin (PLTX) analogue identified in the benthic dinoflagellate *Ostreopsis cf. ovata* from the Mediterranean area. Humans can be exposed to OVTX-a mainly through inhalation of marine aerosol and/or skin contact with seawater during dinoflagellates' blooms, with possible threat to public health. Despite the hazard posed by PLTX has been extensively characterized, very few data are currently available for OVTX-a. Hence, this study was aimed at assessing the cutaneous *in vitro* effects of OVTX-a using spontaneously immortalized HaCaT keratinocytes.

The effects of OVTX-a ($1x10^{-16}$–$1x10^{-7}$ M) in HaCaT cells were compared to those of the reference toxin (PLTX), in terms of cell viability, cell necrosis, reactive oxygen species (ROS) production and mitochondrial depolarization. After 4 h exposure, OVTX-a induced a concentration-dependent cell viability reduction ($EC_{50}=8.3x10^{-9}$ M), with one order of magnitude lower potency than that of PLTX ($EC_{50}=3.7x10^{-10}$ M). Accordingly, OVTX-a induced a concentration-dependent increase of cell necrosis with a potency lower than that of PLTX. Moreover, despite OVTX-a increased ROS production similarly to PLTX, it caused a lower mitochondrial depolarization in keratinocytes with respect to the reference toxin. Then, to investigate the possible mechanisms involved in OVTX-a cytotoxicity, the same cellular parameters were assessed in presence of ouabain (OUA, $1.0x10^{-5}$ M) as inhibitor of Na^+/K^+ ATPase, the molecular target of PLTX, or diphenyliodonium chloride (DPI, $5.0x10^{-6}$), a non-specific inhibitor of flavoprotein-based enzymes, known to be involved in PLTX-induced oxidative stress. On the whole, results suggested that OVTX-a and PLTX share the same molecular target and mechanism of cytotoxicity.

In conclusion, this study provided a contribution in the characterization of the toxic effects of OVTX-a in skin keratinocytes. Although less potent than PLTX, the OVTX-a cytotoxic effects at nanomolar concentrations after a short exposure time rise some concern for humans exposed to this toxin during *Ostreopsis* blooms.

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P21-65
Androgenic and anti-androgenic effects of nanoplastics

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Excessive use of plastic and inappropriate disposal of plastic waste have led to a massive accumulation of plastic particles in the environment. Micro- and nanoplastics can be released into environment directly or derived from larger plastic items by mechanical, biological or chemical degradation. Due to their small size and large surface area, nanoplastics can penetrate various barriers and adsorb chemical substances onto their surface. This property represents additional risk for toxic effects on marine and soil organisms but also human health. [1] Although the presence of plastic particles in the human body was confirmed, the effect of these particles and their mixtures on the human endocrine system has not been clarified. [2]

This study aimed to evaluate toxicity and endocrine disrupting activity of 8 different plastic nanoparticles (PNPs) and their mixtures on cell line AREcoScreen GR KO M1. This cell line was developed to test the effects of various chemicals on the androgen receptor (AR) activity. In order to examine the influence of polymer type and nanoparticle size on their ability to interact with androgen receptor, we used polystyrene nanoparticles (PSNPs) sized 50 nm, 150 nm, 350 nm and polyethylene nanoparticles (PENPs) sized 50 nm and 350 nm. Additionally, we included polypropylene nanoparticles (PPNPs) sized 50 nm and 180 nm and polyethylene terephthalate nanoparticles (PETNPs) sized 80 nm. To examine the effects of PNPs complex mixtures, we combined nanoparticles of approximately the same size and used the same concentration of each polymer to prepare the mixtures. Cytotoxicity of PNPs individually and in mixtures was tested using MTS assay prior to any experiments on AR activity. To determine whether used PNPs and their mixtures were androgen receptor agonists or antagonists, transactivation assay was performed in accordance with the