



SETAC EUROPE
23RD ANNUAL MEETING
GLASGOW, UK
12 – 16 MAY 2013

abstracts
book



BUILDING A BETTER FUTURE:
RESPONSABLE INNOVATION AND
ENVIRONMENTAL PROTECTION



available studies that could potentially meet EPA's study quality criteria as critical studies in the IRIS program. In addition, the authors have performed dose-response modeling using EPA's benchmark dose model to identify *point of departure* doses and composite uncertainty factors to derive the five toxicological reference values in accordance with standard EPA policy and guidance. The authors have also evaluated alternative approaches to characterize the uncertainties in deriving the above toxicological reference values. In so doing, the authors have identified the toxicological reference values likely to be presented in EPA's draft document. This research will be used to prepare detailed comments to the EPA docket on EPA's toxicological assessment of BaP. If the document is released as scheduled, the presentation will compare the authors' proposed toxicological reference values to the EPA's proposed values and critically evaluate the scientific merit of the EPA's toxicological assessment of BaP. Specifically, the presentation will summarize the comments prepared for and submitted to the EPA docket. Additionally, the presentation will discuss the implications for the regulated community of the proposed toxicological reference values coupled with the Relative Potency Factors (RPFs) that are due to be issued by EPA in final form in late 2013. Updated whole mixture validation exercises of carcinogenic endpoints will also be presented.

WE236

Toxicity of mixture herbicides used in sugarcane crops to juvenile tilapia (*Oreochromis niloticus*)

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Tilapia (*Oreochromis niloticus*) is the main specie cultivated in São Paulo State, Brazil, due to its high aquaculture potential. The predominant culture in this region is the sugarcane, which demands the intensive use of equipment and pesticides that have the potential to contaminate water bodies adjacent to the cultivation areas. Herbicides ametryn and tebutiuron are commonly used in mixture in the spray tank in sugarcane crops, although it is prohibited by law. Possible interactions between different herbicides used simultaneously are not understood and the impact of this practice on non-target organisms is not well known. In this work we evaluated the prolonged toxicity (14 days) of herbicides ametryn + tebutiuron (control, 0.1076 e 1.076 mg L⁻¹ active ingredient) to juvenile tilapia. Both herbicides were used in the form of commercial products in concentrated suspension containing 50% a.i.. Tests were conducted in duplicate, corresponding to 1/100 e 1/10 of LC_{50-96h} previously determined. Fish (Ls = 9.98 ± 0.56 cm; Wt = 33.48 ± 6.15 g) (n=36) were fed *ad libitum* and maintained in tanks of 115L with aeration, in a density around 3.8 g L⁻¹, in room with temperature (26.0 ± 2 °C) and photoperiod (16:8h, light: dark) control. At seven and 14 days of exposure, fish were sacrificed (benzocaine solution 8%) and blood and liver were collected. Blood was drawn by caudal puncture, with the help of a needle and syringe previously heparinized. The blood specimens were assayed for: number of erythrocytes (RBC), counted in a Neubauer chamber; hematocrit (Ht) by the microhematocrit technique; and hemoglobin level (Hb) by the cyanomethemoglobin method. After these procedures, the following RBC indices were calculated: MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration). Assays were performed to analyze enzymatic activities (GST, CAT, GPx and SOD) in fish liver. These samples were stored at -80 °C until enzymatic analysis. The data from this study will allow a better understanding of the mode of action and toxicity of this mixture to tilapia. Also, would be useful for the establishment of maximum concentration limits in water bodies. For various pesticides, these parameters are not yet determined in Brazilian resolutions. Since it is almost impossible to found these pesticides isolated in nature, the real risk would be underestimated by the use of data from a single substance.

WE237

Assessment of lethal and sublethal toxicity of disinfectants mixtures to *Daphnia magna*

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Disinfectants are chemicals with the property to eliminate viruses, bacteria and fungi in a short period of time. These chemicals are increasingly used for clinical and veterinary purposes and also in personal care products. After use they reach the aquatic environment through effluents, forming complex mixtures. Chlorhexidine digluconate (ChD) and benzalkonium chloride (BKC) are among the most common disinfectants. In this work we assessed lethal and sublethal toxicity of binary mixtures of ChD and BKC to the crustacean *Daphnia magna*. Lethal and sublethal effects were determined as immobilization and feeding inhibition, respectively. Both disinfectants are highly toxic to the daphnids, with median effective concentrations in the order of micrograms per liter. The

appropriate prediction model for the lethal and sublethal toxicity of the mixtures is presented, using the model that best fits the data (independent action and concentration addition).

WE238

Toxicity of carbendazim and triclosan on *Daphnia magna* and species sensitivity distribution on aquatic organisms

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Pesticides have become an indispensable tool for the sustainability of Humanity through crop protection and pest control. Carbendazim and triclosan are examples of widely used chemical compounds: carbendazim is a fungicide largely used in agriculture and triclosan has a biocide action and it's used in a variety of personal care products (e.g. toothpaste, shampoos, cosmetics, etc). However, these compounds end up in the aquatic system, affecting negatively a large number of organisms. This work aimed to assess the effect of carbendazim and triclosan on the freshwater cladoceran species *Daphnia magna* and to evaluate the ecological risk to aquatic ecosystems by constructing Species Sensitivity Distributions (SSDs) based on organisms' traits. Different bioassays were performed: acute immobilisation test, feeding inhibition test and reproduction test (where the number/size of broods and number of aborted eggs per animal; and the body length of the parent animal were measured). SSDs and the hazardous concentration at 5% (HC₅) were estimated considering different organisms traits. A decrease in the number of neonates per animal and increase in the number of aborted eggs (reproduction test) was noticed with increasing concentrations of carbendazim and triclosan. Also, in the feeding inhibition test, a decrease on feeding rate was observed, as well as mortality as the concentrations increase. SSDs revealed to be a promising method to assess ecological risk, however further information about species traits is needed to increase the accuracy of this approach.

WE239

Waste water harmful effect detection by using biological methods – bioassays and benthic macroinvertebrate metrics

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One of the main reasons of the Baltic Sea pollution is leaching waste water from the waste water treatment plants along the sea and also from inland. With HELCOM Baltic Sea Action Plan the Baltic Sea countries have committed themselves to achieve Baltic Sea with life undisturbed by hazardous substances hence reaching good environmental quality status. The aim of this study was to evaluate harmful effect of biologically treated waste water (WW) from four WW treatment plants (WWTP) by using several bioassays and benthic macroinvertebrate metrics. Samples for acute bioassays to detect WW harmful toxic effect were collected 5 times (from May 2011 to May 2012). Toxicity was determined by using 4 different acute toxicity standard methods: freshwater - *Daphnia magna* immobilization test (EN ISO 6341:1996), luminescent bacteria inhibition test (*Vibrio fischeri* LVS EN ISO 11348-3:1998), freshwater algae test (*Desmodesmus communis* LVS EN ISO 8692:2005) and saltwater - *Artemia salina* test (ArtoxKit M standard method). To calculate benthic invertebrate metrics in order to detect WW negative impact on benthic fauna of watercourses where WW are discharged, zoobenthos samples were collected in May 2012 50 m upstream and downstream from the WW discharge place by modified AQEM (A comprehensive method to assess European streams using benthic macroinvertebrates) method. The acquired bioassay results evidences about periodic WW acute toxicity. Main reasons for that are seasonal changes, WW day and night inflow intensity, abundance of hazardous substances, active sludge activity etc. Comparing testorganism sensitivity of applied bioassays it can be assumed that the most sensitive testobject against biologically treated WW is *D.magna*, but the most toxicoresistant organism – *V.fischeri*. *D.communis* and saltwater organism *A.salina* can be characterized as intermediate sensitive. Besides in *V.fischeri* and *D.communis* tests it was possible to observe bioluminescence and growth stimulation evidencing about more organic and inorganic than toxic substance abundance. It proves that in these cases WW can promote or cause eutrophication. Benthic macroinvertebrate metrics showed similar results in two WWTP cases - most of the metrics evidenced about organic pollution increase downstream the WW discharge place. Study revealed that it is necessary to improve treatment processes in Latvia's WWTPs. This study was performed in the frame of ESF projects 2009/0226/1DP/1.1.1.2.0/09/APIA/VIAA/080.

WE240

Predictions gone wrong: mixture toxicity of a repellent and a pyrethroid on *Daphnia magna*

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In the past decades, combinatory effects of substances have been of growing interest. Still, knowledge on the "cocktail-effect" of compounds is limited. For the prediction of mixture toxicity, the models of concentration addition (CA) and