Abstract Book

Society of Environmental Toxicology and Chemistry
North America 40th Annual Meeting

Great Together: Separate Challenges and Collective Solutions
3–7 November 2019 | Toronto, Ontario, Canada

SETAC®

We measured mitochondrial oxygen consumption after exposure in order to determine the effect in the ATP production and in mitochondrial complexes I and II. We also determined the effect of TFM and metabolites on the mitochondrial membrane permeability by measuring the transmembrane potential after exposure. Mitochondrial bioenergetics were heavily affected when exposed to increasing concentrations of TFM, methyl-phenol, and amino methyl-phenol at the different protein complexes examined, which suggests that the mode of action of these compounds goes beyond the uncoupling of complex V (ATP-synthase) at the end of the electron transport chain but at an earlier stage. The transmembrane potential was greatly reduced by TFM and methyl-phenol, but it was not affected by the amino metabolites.

RP063 Effects of the gill microenvironment on toxicant speciation and uptake by fishes: A case study using the piscicide TFM

M.P. Wilkie, S.L. Hepditch, L. Tessier, O. Birceanu, Wilfrid Laurier University / Biology

Invasive sea lamprey (*Petromyzon marinus*) populations are controlled using a piscicide, 3-trifluoromethyl-4-nitrophenol (TFM). Applied to infested streams, TFM targets larval lampreys, which have a low capacity to detoxify the agent. However, non-target adverse effects and mortality can occur during TFM applications, particularly following decreases in water pH. A weak acid, with a pKa of 6.07-6.38, total TFM (TFM_{tot}) exists as un-ionized TFM-OH or ionized TFM-O^-. As water pH decreases, TFM-OH, the bioavailable form of TFM_{tot}, increases leading to greater uptake and toxicity. Similarly, TFM toxicity is higher in low alkalinity waters, but the reasons are unclear. Our goal was to determine how water alkalinity and the chemistry of the gill microenvironment, defined as the expired water crossing the gills, influenced TFM uptake and TFM-OH bioavailability to lake sturgeon (*Acipenser fulvescens*) and rainbow trout (*Oncorhynchus mykiss*). Using radio-labelled TFM (^{14}C-TFM), we observed that TFM uptake by lake sturgeon was highest at pH 6.5 versus pH 9.0, confirming that uptake takes place by diffusion as un-ionized TFM-OH. Similarly, TFM uptake was greatest at low (~ 50 mg $CaCO_3$ L^{-1}) compared to moderate (~ 150 mg $CaCO_3$ L^{-1}) and high alkalinity (~ 250 mg $CaCO_3$ L^{-1}). As water pH was circumneutral (pH 7.8-8.2), differences in bulk water pH could not explain our findings. Instead, we hypothesized that greater acidification of the gill microenvironment resulted in higher TFM-OH bioavailability in low compared to higher alkalinity water. To test this hypothesis, trout were implanted with opercular catheters, allowing us to measure the pH of water expired across the gills (the microenvironment). At low alkalinity, expired water pH was 1.0-1.5 pH units lower than inspired (bulk) water pH, likely due to acid (H^+) excretion and CO_2 excretion across the gills. Notably, acidification was less at moderate alkalinity, and eliminated at high alkalinity. Thus, lower TFM uptake at higher alkalinities was due to higher water buffering capacity, which reduces or prevents acidification of the expired gill water. Our results demonstrate that opercular catheters, which are easy to install and cause minimal stress to fish, allow accurate measures of water chemistry and toxicant bioavailability within the gill microenvironment. Using this approach, we also show that water-buffering capacity can profoundly affect toxicant bioavailability in the gill microenvironment.

RP064 Biochemical biomarkers in shrimp (Litopenaeus vannamei) exposed to chlorpyrifos

B.E. Jaramillo-Colorado, University of Cartagena / Chemistry Program; E. Duarte-Restrepo, University of Cartagena / Agrochemical Research Group / Chemistry Program; L. Duarte-Jaramillo, University of Cartagena / Agrochemical Research Group

Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate) residues were detected in sediments of Cartagena Bay. This pesticide comes from the Dique channel (Magdalena River) and spills of industries of the Mamonal sector In Cartagena, Colombia. Chlorpyrifos (CPF) is an organophosphosphorus pesticide (OP) extensively applied to control pests and agricultural practices, but they also adversely affect non-target fauna. It is the second largest selling OP and found to be more toxic to the marine organisms than organochlorine compounds. The effect of this OP on the shrimp (Litopenaeus vannamei) was evaluated. LC50 (96-hours) was determined. Lipid oxidation levels (LPO) and activities of catalase (CAT), glutathion peroxidase (GPx), glutathion-S-transferase (GST) were assessed on the muscle, hepatopancreas and gills from adults exposed to two sublethal CPF concentrations (0.7 and 1.3 µg/L) for four days. Furthermore, acetylcholinesterase (AchE) inhibition was determined in the brain. LC50 (96-hours) was 2.10 µg/L of CPF. Shrimp exposed to two sublethal CPF concentrations showed an increase of CAT activity in the three tissues, a decrease of AchE activities in the brain and an increase of GST activity in the hepatopancreas, while LPO increased in all tissues. These results show that L. vannamei could be used as biomarkers to monitor OP pesticides in aquatic environments.

RP065 Assessing the impacts of methoxychlor exposure on the viability, reproduction, and behavior of the seminole ramshorn snail (Planorbella duryi)

T.E. Frankel, University of Mary Washington / Earth and Environmental Sciences; M. Bohannon, University of Maryland / Environmental Science and Technology; J. Frankel, Howard University / Biology

In this study, the effects of short-term methoxychlor exposure on the viability, reproduction, and locomotor behavior of adult Seminole ramshorn snails (*Planorbella duryi*) was assessed. To examine impacts on viability and behavior, individual snails were exposed to a water control, vehicle control, 12.5, 50, 100, 250, 500, or 1000 μg/L of methoxychlor for 48hrs and differences in mortality and locomotor behavior were examined. To determine impacts on reproduction, pairs of snails were exposed to a vehicle control, 12,5, 50, 100, and 250 μg/L of methoxychlor for 9 days and the number of egg clutches and viable embryos laid quantified every 24hrs. To verify nominal vs. actual concentrations, water samples were collected and methoxychlor concentrations determined using gas chromatography. Complete mortality was observed in the 500 μg/L and 1,000 μg/L treatments after 48hrs and in the 250 μg/L treatment after 9 days. Significant decreases in the number of egg clutches were observed in all treatments, and the number of embryos laid decreased starting in the 25 μg/L treatment. Decreases in average speed, mobile speed, and total distance travelled as well as a significant increase in frozen events were also observed. Our results suggest that methoxychlor exposure causes detrimental effects on several non-lethal endpoints in an alternative model aquatic invertebrate species and that the analysis of locomotor behaviors serves as a reliable, sensitive endpoint for future ecotoxicology testing.

RP066 Effects of pesticides with different mechanism of toxicity in the freshwater prawn Macrobrachium borellii

S.M. Lavarías, K. Colpo, E. Ambrosio, Instituto de Limnología "Dr. Raúl A. Ringuelet" (CCT- La Plata CONICET –UNLP); S. Landro, Museo Argentino de Ciencias Naturales (CONICET); A. Rodrigues Capítulo, Instituto de Limnología "Dr. Raúl A. Ringuelet" (CCT- La Plata CONICET –UNLP) / Instituto de Limnología Dr Raúl A Ringuelet CCT La Plata CONICET UNLP; F. Arrighetti, Museo Argentino de Ciencias Naturales (CONICET)

Synthetic pyrethroids like cypermethrin (CYP) are extensively applied for control of agricultural pests and disease vectors. However due to its harmful consequences to non-target organisms, the bioinsecticides represents a benign and desirable alternative to the environment. Among them the biological insecticide produced from *Bacillus thuringiensis israelensis* (*Bti*) is mainly used for the control of mosquito vectors of diseases like dengue. Anyway, pest organisms have developed resistance mechanisms to such kind of insecticides so last generation pesticides, such as the tetramic acid spirotetramat (STM), have recently emerged. Due to the limited information available on the effect of these compounds on non-target species, the effect of CYP, *Bti* and STM on the freshwater prawn *Macrobrachium borellii* was analyzed. Initially, the LC_{50}-96 h was determined in adult prawns (males and females in non-vitellogenic state), by serial dilutions of the three insecticides. A negative control without insecticides was included. Then, in order to determine metabolic disorders that could be

used as biomarkers of pollution, prawns were exposed to sublethal concentrations of CYP (0.006 and 0.02 µg /L), *Bti* (0.04 and 0.4 mg /L) and STM (0.5 and 1.7 mg/L) for 4 days. The levels of lipid peroxidation (LPO) and protein oxidation (OP), as well as the presence of histopathological changes were evaluated in the hepatopancreas. The LC_{50}-96 h values were 0.8 and 8.2 mg /L for *Bti* and STM respectively, and 0.12 µg /L for CYP. All three insecticides significantly affected the LPO and OP levels in this organ ($p < 0.05$). Pesticides caused histopathological alterations in the hepatopancreas of the exposed prawns, such as atrophy in the epithelium of the digestive tubules, necrosis of the epithelial cells, and the infiltration of hemocytes into the connective tissue between the intertubular space. This study showed that *M. borellii* is less sensitive than other crustaceans to CYP. In addition they are the first results of sensitivity to *Bti* and STM in this taxonomic group. Considering that the hepatopancreas is the main organ for toxicant metabolism in crustaceans, these results indicate that oxidative status in *M. borellii* is very sensitive to these insecticides. Therefore, this prawn could be proposed as useful bioindicator to monitor freshwater environments that are exposed to these pollutants.

RP067 A comparison of alternative 96-hr Hyalella azteca water exposure protocols for the sensitivity to the pyrethroid bifenthrin

S.L. Clark, S. Vasquez, Pacific EcoRisk; S. Rees, T. Albertson, Caltest Analytical Laboratories; B.C. Jorgenson, Pacific EcoRisk

The Central Valley Regional Water Quality Control Board (California, USA) adopted a pyrethroid basin plan amendment and total maximum daily loading (TMDL) limits in 2018 requiring monitoring by publically owned treatment works, stormwater dischargers, and irrigated agriculture. As the goal of the monitoring is to determine the extent to which pyrethroids cause water column toxicity, water samples are analyzed for pyrethroids, organic carbon, and acute toxicity to *Hyalella azteca*in 96-hr bioassays. In order to appropriately determine if toxicity is due to pyrethroids, sufficiently sensitive methods must be applied and an environmentally relevant, yet cost effective, toxicity testing protocol must be used; it is imperative that the toxicity test design does not mask the toxicity of these extremely hydrophobic pesticides. There are currently two toxicity tests based on limited guidance in the 2002 EPA acute testing manual that are under consideration: the method applied for Surface Water Ambient Monitoring Program (SWAMP) studies and a method refined by the Southern California Coastal Water Research Program (SCCWRP) during a stormwater inter-calibration study. Both methods require static renewal testing with solution renewals at 48 hours. The SWAMP method includes the addition of food throughout the testing, whereas the SCCWRP method requires feeding 2 hrs before solution renewal. As demonstrated by others, substantial loss of pyrethroids to the walls of the bioassay test chamber is a significant concern. This loss, amplified by the 48-hour renewal test design could result in the SWAMP and SCCWRP protocols underestimateing pyrethroid toxicity. Furthremore, the inclusion of food in the test chambers throughout testing (i.e., SWAMP method) could reduce the dissolved phase pyrethroids to which the organisms are exposed. To address this concern, a study was performed in which these two toxicity methods were compared to a modified SCCWRP protocol with daily solution renewals and feeding 2 hours prior to each renewal, with bifenthrin used as the toxicant. All testing was performed concurrently using the same bifenthrin stock solutions prepared daily. Stock solutions and representative samples collected during the testing were analyzed for bifenthrin using negative chemical ionization, GC/MS with selected ion monitoring (EPA 625.1M). The results of this testing will be presented, along with the ramifications for monitoring under the pyrethroid TMDL.

RP069 Assessing the impacts of sulfoxaflor exposure on the viability, growth, behavior, and hematocrit of the seminole ramshorn snail (Planorbella duryi)

T.E. Frankel, F. Hodges, University of Mary Washington / Earth and Environmental Sciences

Sulfoxaflor, a sulfoximine insecticide, has seen increased utilization as an effective tool against sap-feeding insect species that have developed resistances to other control agents. However, few studies have been conducted to examine the impacts of sulfoxaflor exposure on viability and non-lethal endpoints in freshwater aquatic invertebrates. As such, this study examined the effects of sulfoxaflor exposure on the viability, growth, locomotor behavior, and hematocrit of juvenile seminole ramshorn snails (*Planorbella duryi*). Individual juvenile snails (> 1 day post hatch) were exposed to a water control, vehicle control, 0.1, 1, 10, 100, and 1000 μg/L of sulfoxaflor for six weeks using a weekly static replacement exposure method. Mortality, defined as a lack of response to footpad contact, was assessed daily. To examine the impacts of sulfoxaflor on growth, a photograph of each snail was taken weekly and the shell height and width analyzed using ImageJ. A 15 minute video recording of each snail was obtained on weeks 3, 4, 5, and 6 and differences in locomotor behaviors assessed using automated behavioral analysis software. At the end of the exposure period, hemolymph from each individual was collected in heparin-treated glass capillary tubes, centrifuged, and packed cell volume determined using a microhematocrit capillary tube reader. While this study is still ongoing, we expect to observe significant decreases in growth, locomotor behaviors, and packed cell volumes in individuals exposed to sulfoxaflor in a dose-dependent manner. Findings from this study will help to elucidate the effects of sulfoxaflor contamination on aquatic invertebrates while also providing support for future studies to examine the presence and concentrations of sulfoxaflor in aquatic ecosystems.

RP070 Lethal and sub-lethal effect of copper sulphate pentahydrate on two successive generations of Culex pipiens mosquito (Diptera: Culicidae)

N. Amer, S.P. Lawler, Universtiy of California, Davis / Entomology and Nematology; R.E. Connon, University of California, Davis / Anatomy, Physiology & Cell Biology / School of Veterinary Medicine

The *Culex pipiens* mosquito is an important public health vector of many diseases. It usually inhabits large ponds and sometimes is found in small water bodies. Copper may enter water when used as a biocide in antifouling paint formulations or from agricultural and urban uses. Copper sulfate is used as a fungicide, algaecide and for root treatment, and is especially widely used in rice fields in California. We studied lethal and sub-lethal effects of copper sulfate pentahydrate on the immature stages of two successive generations of *Cx. pipiens* using laboratory toxicity testing. Larval mortality rate increased with increased concentrations during both generations. During the 1st generation, mortality rates increased gradually from control to 100% at 1 mg/l. The 2nd generation showed a marked increase in mortality rates of the larval stage relative to generation one, especially at the higher concentrations of 0.25 and 0.5 mg/l. The larval LC_{50} level of the second generation was 40% lower than the first. Copper sulfate exposure delayed development in both generations, and pupation rate also decreased with increased concentrations in both generations. This study showed higher sensitivity of *Cx. pipiens* to copper toxicity than other mosquito species. The higher sensitivity of the 2nd generation is a relatively novel finding because multi-generation studies are rare in comparison to short-term tests. More such studies are needed to determine if exposure of the parents to toxicants is usually detrimental to offspring.