EXHIBIT 1

EARTHJUSTICE

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Exhibit 1: References to Recent (2006–2008) Studies on the Environmental Fate and Aquatic Toxicity of Chlorpyrifos, Diazinon, and Malathion

Chlorpyrifos

1) Ashauer R., A. Boxall, and C. Brown, 2007, Modeling combined effects of pulsed exposure to carbaryl and chlorpyrifos on Gammarus pulex. Environ Sci Tech 41(15): 5535–41.

Abstract: Aquatic risk assessment can be improved if we are able to quantitatively predict the effects resulting from sequential pulsed exposure to multiple compounds. We evaluate two modeling approaches, both extended to suit multiple compounds, the semi-mechanistic threshold damage model (TDM), and a model based on time-weighted averages (TWA). The TDM predicts that recovery of damage to Gammarus pulex from exposure to chlorpyrifos takes longer than that from exposure to carbaryl and consequently that the sequence of exposure matters. We measured survival of the freshwater invertebrate Gammarus pulex after sequential pulsed exposure to carbaryl and chlorpyrifos. Two groups of organisms were exposed to a first pulse of either carbaryl or chlorpyrifos for 1 day and then, after a recovery period of two weeks, to a second pulse with the other compound. The comparison of mortalities caused by each pulse, as well as combined mortalities in both treatments, show that the sequence of exposure to pulses of contaminants does indeed matter. Previous exposure to chlorpyrifos leads to significantly increased mortality from subsequent pulses of carbaryl, but not the other way round. The TDM facilitates a process-based ecotoxicological explanation by simulating the recovery dynamics and outperforms the TWA model.

2) Ashauer, R., A. Boxall, and C. Brown, 2006, Uptake and Elimination of Chlorpyrifos and Pentachlorophenol into the Freshwater Amphipod Gammarus pulex, Arch.Environ.Contam.Toxicol. 51(4):542-548.

Abstract: Uptake and elimination rates were determined for chlorpyrifos (CPF) and pentachlorophenol (PCP) in the freshwater amphipod Gammarus pulex. Internal concentrations of the two pesticides were measured over a three-day exposure phase and a subsequent three-day elimination phase. Rate constants were obtained by fitting measured internal concentrations to a one-compartment single first-order model. The uptake rate constants were 747+/-61 [L kg-1 day-1] for CPF and 89+/-7 [L kg-1 day-1] for PCP. The elimination rate constants were 0.45+/-0.05 [day-1] for CPF and 1.76+/-0.14 [day-1] for PCP. The resulting bioconcentration factors at steady state were 1660 and 51 for CPF and PCP, respectively. The parameter estimation method and possible variability due to varying lipid content are briefly discussed.

3) Binelli, A., F. Ricciardi, C. Riva, and A. Provini, 2006, New Evidences for Old Biomarkers: Effects of Several Xenobiotics on EROD and AChE Activities in Zebra Mussel (Dreissena polymorpha), Chemosphere 62(4):510-519.

Abstract: The biomarker approach is widely used both in vertebrates and invertebrates for environmental biomonitoring, because it can supply an integrated response for multi-xenobiotics contamination. However, the use of biomarkers requires the identification of every possible variation that can influence the biochemical response, because ecosystems are generally subject to a mixture of pollutants, which can create additive, opposite or competitive effects. In recent years, there has been considerable interest in the use of biomarkers within marine bivalves, while very few data are available for freshwater molluscs. The aim of this research was to investigate changes on EROD and AChE activities in the freshwater bivalve Zebra mussel (Dreissena polymorpha) exposed to different pollutants (Arochlor 1260, CB 153 and 126, pp'DDT, chlorpyrifos, carbaryl) at laboratory conditions, in order to standardize the analytical procedures and to highlight eventual interferences on enzyme activities. Chemical concentrations in the mussel soft tissues were analyzed by GC/MS-MS. Main results showed a significant induction of EROD activity when mussels were exposed to 100 ng/l of PCB mixture of Arochlor 1260 and dioxin-like CB 126, but this congener showed also a clear competitive inhibition after 48 h of exposure. Surprisingly, pp'DDT determined a significant decrease of basal EROD activity after only 24 h of exposure, even if it was not possible to discriminate between the effect of the parent compound and that of its metabolites (DDD, DDE). We also found an interaction between the organophosphate insecticide chlorpyrifos, which does not directly decrease the AChE activity, and terbutilazine. This herbicide increased the biotransformation of the organophosphate compound to its oxidized metabolite (oxon), a much stronger AChE inhibitor. The possible use of the oxime Pyridine-2-Aldoxime Methochloride (2-PAM) to bring back the catalytic activity to basal levels was also demonstrated.

4) Caceres T, He W, Naidu R, Megharaj M. 2007. Toxicity of chlorpyrifos and TCP alone and in combination to *Daphnia caranata*: The influence of microbial degradation in natural water. *Water Res* 41: 4497–4503.

Abstract: The acute toxicity of chlorpyrifos and its principal metabolite 3,5,6-trichloropyridinol (TCP) alone and in combination to a cladoceran, *Daphnia carinata*, was studied in both cladoceran culture medium and natural water collected from a local suburban stream. TCP was found to be more toxic than its parent chemical chlorpyrifos to Daphnia survival in cladoceran culture medium. However, TCP in natural water was not toxic to D. carinata up to 2 microgL(-1). The LC(50) values for chlorpyrifos, TCP and chlorpyrifos+TCP were 0.24, 0.20 and 0.08 microgL(-1), respectively, in cladoceran culture medium. Although the parent chemicals and their degradation products co-exist in natural waters, the existing guidelines for water quality are based on individual chemicals. The results of this investigation suggest that chlorpyrifos and TCP can interact synergistically, additively or antagonistically, resulting in an increase or decrease in the

overall toxicity of the mixture compared to individual compounds. The indigenous microorganisms in natural water could play a significant role in degradation of these compounds thereby influencing their toxicity in receiving waters. This study clearly suggests that the joint action of pesticides and their degradation products should be considered in the development of water quality guidelines. To our knowledge, this is the first study on the interactive effect of chlorpyrifos and TCP to a cladoceran and suggests that these two compounds are non-toxic when present together at concentrations up to 0.12 microgL(-1). However, these compounds together act additively at and above 0.5 microgL(-1) to fresh water invertebrates and therefore pollution with these compounds may adversely affect natural ecosystems.

5) Carriger J F, G M Rand, 2008, Aquatic risk assessment of pesticides in surface waters in and adjacent to the Everglades and Biscayne National Parks: I. Hazard assessment and problem formulation, Ecotoxicology 17:660–679. DOI 10.1007/s10646-008-0230-0.

Abstract: An aquatic risk assessment under the U.S. Environment Protection Agency (EPA) ecological risk framework was conducted for atrazine, metolachlor, malathion, chlorpyrifos, and endosulfan in the C-111 freshwater basin (eastern boundary of the Everglades National Park), northeast Florida Bay, and south Biscayne Bay in South Florida. Based on the use of the hazard quotient approach, measured concentrations of chlorpyrifos and endosulfan in surface waters suggest potential hazards to aquatic organisms and were, therefore, considered as chemicals of potential ecological concern (COPECs). The problem formulation included an overview of the physical/ chemical and environmental fate characteristics and aquatic toxicology of the COPECs. Background surface water exposure concentrations of endosulfan and toxicity data from laboratory and field studies indicate that fish and invertebrate mortality may be a concern when endosulfan is applied in agricultural areas near aquatic ecosystems.

6) Chandrasekara, L.W.H.U., A. Pathiratne, 2007, Body Size-Related Differences in the Inhibition of Brain Acetylcholinesterase Activity in Juvenile Nile Tilapia (Oreochromis niloticus) by Chlorpyrifos and Carbosulfan, Ecotoxicol.Environ.Saf. 67(1):109-119.

Abstract: Influence of body size on inhibition of brain acetylcholinesterase (AChE) activity of juvenile Nile tilapia, Oreochromis niloticus by chlorpyrifos and carbosulfan was investigated concerning its potential use in the biomonitoring of anticholinesterase pesticides in tropical water bodies. Three size groups of fish (fry: 3-4 cm, fingerlings: 6-8 cm, sub-adults: 10-12 cm in total length) were exposed to a series of concentrations of chlorpyrifos (0.5-12 microg L(-1)) or carbosulfan (1-10 microg L(-1)), and concentration-response for inhibition and recovery of the AChE enzyme was evaluated in comparison to the controls at different time points, 2, 6, 10, and 14 d. The AChE activities of the control fish followed the order of decreasing activity, fry>fingerlings>sub-adults. AChE activities of the fry were nearly 2-fold higher than that of the sub-adults. Following 48 h of pesticide exposure, the AChE activity of the three size groups of fish decreased significantly in comparison to the respective controls in a concentration-dependent manner. The activity

was greatly inhibited in the fry (39-85%) compared to sub-adults (18-47%) exposed to the most of the similar concentrations of the pesticides. Median effective in vivo inhibition concentrations (48 h IC50) of chlorpyrifos for fry, fingerlings, and sub-adult stages were 0.53, 0.75, and 3.86 microg L(-1), respectively, whereas the corresponding values for carbosulfan were 3.37, 7.02, and 8.72 microg L(-1). When fish were maintained in the initial pesticide medium for 14 days, AChE activity restored gradually depending on the initial pesticide exposure concentration and the size group of the fish. Results indicate that brain AChE of Nile tilapia is a promising biomarker for assessment of anticholinesterase pesticide contaminations in water. However, body size of Nile tilapia should be taken into account when using this biomarker in biomonitoring programmes.

7) Colville A, Jones P, Pablo F, Krassoi, F, Hose G, Lim R, 2008, Effects of chlorpyrifos on macroinvertebrate communities in coastal stream mesocosms, Ecotoxicology 17(3): 173–180.

Abstract: This study measured the effects of a single pulse of chlorpyrifos at nominal concentrations of 1 and 10 microg/l on the macroinvertebrate community structure of a coastal stream mesocosm system. Analysis of data using Principal Response Curves (PRC) and Monte Carlo tests showed significant changes in the treated stream mesocosms relative to that of the controls. These changes in the macroinvertebrate assemblages occurred within 6 h, and persisted for at least 124 days after dosing. Significant community-level effects were detected at the lowest concentration on days 2 and 16 post-dosing, giving a no-observed effect concentration (NOEC(community)) of 1.2 microg/l (measured). The mayflies Atalophlebia sp. and Koorrnonga sp., Chironomidae and Acarina were all sensitive to chlorpyrifos and decreased in abundance in treated mesocosms after dosing. The fauna of these coastal stream mesocosms showed similar sensitivity to chlorpyrifos with that of other reported studies, but there was no evidence of recovery after 124 days.

8) Cooper, N.L., and J.R. Bidwell, 2006, Cholinesterase Inhibition and Impacts on Behavior of the Asian Clam, Corbicula fluminea, After Exposure to an Organophosphate Insecticide, Aquat. Toxicol. 76(3/4):258-267.

Abstract: This study assessed the effects of exposure to an insecticide formulation containing the organophosphate, chlorpyrifos on cholinesterase activity, siphoning and burrowing ability in the Asian clam, Corbicula fluminea. Clams were exposed to concentrations of the pesticide ranging from 0.05 to 50 mg/L (as chlorpyrifos) in a series of 96-h static bioassays. Those organisms exposed to pesticide concentrations at or above 3.13 mg/L avoided exposure through valve closure during the 96-h test period, with no resultant effects on cholinesterase activity or behavior. Similarly, no effect was observed at low-test concentrations ranging from 0.05 to 0.1 mg/L, even though clams actively siphoned in these treatments. Asian clams exposed to the mid-range of concentrations, 0.5-1.0 mg/L, experienced a significant reduction in cholinesterase activity and a reduced capacity to burrow into the substrate. While these data indicate that cholinesterase activity

in C. fluminea could be used as a biomarker of organophosphorous pesticide exposure, valve closure can clearly influence exposure profiles and biomarker response.

9) Key, P.B., and M.H. Fulton, 2006, Correlation Between 96-h Mortality and 24-h Acetylcholinesterase Inhibition in Three Grass Shrimp Larval Life Stages, Ecotoxicol. Environ. Saf. 63(3):389-392.

Abstract: Three life stages of larval grass shrimp were tested to determine whether acetylcholinesterase (AChE) activity expressed as 24-h sublethal effect endpoints (EC20 and EC50) could be used to predict 96-h mortality (lowest observable effect concentration (LOEC) and LC50) for shrimp exposed to three organophosphate insecticides. With regard to mortality, newly hatched larvae and 18-day-old larvae were the most sensitive in the malathion and azinphos-methyl exposures. In the chlorpyrifos exposures, newly hatched larvae and postlarvae were the most sensitive life stages. Results of the 24-h AChE inhibition tests showed that newly hatched larvae were generally more sensitive in the three organophosphate exposures. A regression analysis of the EC50's and LC50's yielded the strongest correlation with R2=0.987 (correlation coefficient=0.994 and 95% confidence intervals 0.969-0.999). The LOEC/EC20 relationship yielded R2=0.962. For these grass shrimp life stages and pesticides, sublethal effect endpoints could be used as a predictor of 96-h mortality.

10) Luo, Y, Zhang, X, Ficklin, LX, Zhang, M, 2008, Dynamic modeling of organophosphate pesticide load in surface water in the northern San Joaquin Valley watershed of California, Environ Pollut, in press, doi: 10.1016/j.envpol.2008.04.005.

Abstract: The hydrology, sediment, and pesticide transport components of the Soil and Water Assessment Tool (SWAT) were evaluated on the northern San Joaquin Valley watershed of California. The Nash-Sutcliffe coefficients for monthly stream flow and sediment load ranged from 0.49 to 0.99 over the watershed during the study period of 1992-2005. The calibrated SWAT model was applied to simulate fate and transport processes of two organophosphate pesticides of diazinon and chlorpyrifos at watershed scale. The model generated satisfactory predictions of dissolved pesticide loads relative to the monitoring data. The model also showed great success in capturing spatial patterns of dissolved diazinon and chlorpyrifos loads according to the soil properties and landscape morphology over the large agricultural watershed. This study indicated that curve number was the major factor influencing the hydrology while pesticide fate and transport were mainly affected by surface runoff and pesticide application and in the study area.

11) Matozzo, V., A. Tomei, and M.G. Marin, 2006, Effects of 4-Nonylphenol (Xenoestrogen) and Chlorpyrifos (Organophosphorus Pesticide) on Acetylcholinesterase Activity in the Clam Tapes philippinarum, Fresenius Environ.Bull. 15(8a):710-714. NOT AVAILABLE ONLINE.

Abstract**:** Measurement of acetylcholinesterase (AChE) is widely used as a biomarker of exposure to neurotoxic compounds in aquatic environments. In the present study, AChE activity was monitored in the clam Tapes philippinarum in the Lagoon of Venice, with the aim of evaluating the possible exposure of animals to neurotoxic compounds. Two sampling strategies were followed. In the first case, temporal and spatial variations of AChE activity were measured in clams collected seasonally (from October 2003 to June 2004) in 3 sites of the Lagoon: Campalto (site 1), near a sewage treatment plant, Marghera (site 2), a highly polluted area, and Poveglia (site 3), a reference site. The condition index (CI) of clams was also calculated. In the second case, AChE activity was measured in clams collected at the end of seasonal samplings in 12 sites located throughout the Lagoon: areas licensed for clam farming (sites 5 to 11), nearby canals characterised by waste waters from agricultural areas (sites 4, 12, 13, 14), and a reference site outside the Lagoon (site 15). Results revealed a significant reduction in AChE activity in clams from Marghera in October, January and April, indicating their probable exposure to neurotoxic compounds originating in the industrial zone of Porto Marghera. A seasonal trend in AChE activity was also observed, enzyme activity being higher in January and lower in June. Clams from Campalto generally had higher CI values than those from Marghera and Poveglia. However, no particular correlation between AChE activity and CI was found. Lastly, marked differences in AChE activity were recorded when comparing enzyme activity of clams from various sites in the Lagoon of Venice with those of clams collected at site 15. Significant reductions in AChE activity were observed in animals collected in both nearby canals and licensed areas, indicating the homogeneous spatial distribution of potentially neurotoxic compounds throughout the Lagoon. It is probable that sediment mobilisation caused by clam harvesting and the passage of shipping plays an important role in resuspension of persistent contaminants having neurotoxic activity.

12) F. Pablo, F.R. Krassoi, P.R.F. Jones, A.E. Colville, G.C. Hose and R.P. Lim, 2008, Comparison of the fate and toxicity of chlorpyrifos—Laboratory versus a coastal mesocosm system, Ecotox Environ Saf 71(1): 219–229.

Abstract: The widespread use of chlorpyrifos for pest control in urban and rural environments poses a risk of contamination to aquatic environments via runoff, spray drift or spillage. The aim of this study was to assess the fate of chlorpyrifos and its toxicity to common freshwater invertebrates in the laboratory and in stream mesocosms. Chlorpyrifos was rapidly lost from the test systems but the rates of loss varied considerably, such that losses in the mesocosms could not be reliably predicted from the static laboratory studies. This was likely due to the mass transport of chlorpyrifos from the mesocosm via stream flow. Chlorpyrifos was acutely toxic to all invertebrates tested with the cladoceran species (laboratory 48 h LC_{50} values 0.07–0.10 μg L^{-1}) being most sensitive. Despite the differences in the dynamics of chlorpyrifos in the laboratory and mesocosm systems, the sensitivities of the mayfly *Atalophlebia australis* and the cladoceran *Simocephalus vetulus* were similar in the 2 systems.

13) Padovani L, Capri E, Trevisan M. 2004, Landscape-level approach to assess aquatic exposure via spray drift for pesticides: a case study in a Mediterranean area. Environ Sci Technol. 15;38(12):3239-46.

Abstract: The development of methods to extract information from landscape analysis to refine risk assessment is becoming increasingly important. This paper presents results from a pesticide surface water exposure assessment at the watershed scale, based on a combination of edge of field studies, large-scale monitoring studies, and modeling activities with GIS-based landscape analysis methodologies covering an area of approximately 3200 ha surrounding the Simeto River in Sicily (Italy). The dynamic behavior of the pesticide chlorpyrifos-methyl was modeled in two different steps: calculation of the fraction of the application rate that is deposited beyond the field edge and simulation of the fate and persistence of the pesticide in the aquatic environment. Drift loads showed high spatial variability. Considering spray drift deposition as a fraction of the pesticide application rate, 60% of the results were < or = 0.02 (equal to 0.04 mg/m2). Only 8.5% of the results were above 0.5. The highly variability of the landscape factors was reflected in the results. More than 60% of the predicted pesticide concentrations were less than the limit of quantification (0.05 microg/L), affecting about 75% of the total length of the river tract analyzed. Predicted pesticide concentrations were higher than 0.1 microg/L in 23% of cases, but this corresponded to an insignificant portion of the river (1.2% of the total length). These results suggest that management options, such as increased no-spray zones, could provide further protection for surface water. These could be modeled to illustrate their overall impact. As an alternative, the introduction of a 20-m no-spray zone clearly reduced potential exposure, and 92% of the water body was protected. Estimated data are in agreement with data collected during a field monitoring study.

14) Patra, R.W., J.C. Chapman, R.P. Lim, and P.C. Gehrke, 2007, The Effects of Three Organic Chemicals on the Upper Thermal Tolerances of Four Freshwater Fishes, Environ.Toxicol.Chem. 26(7):1454-1459.

Abstract: The upper temperature tolerance limits of four freshwater fish species, silver perch Bidyanus bidyanus, eastern rainbowfish Melanotaenia duboulayi, western carp gudgeon Hypseleotris klunzingeri, and rainbow trout Oncorhynchus mykiss, were determined using the critical thermal maximum (CTMaximum) method. The CTMaximum tests were carried out with unexposed fish and fish exposed to sublethal concentrations of endosulfan, chlorpyrifos, and phenol to determine whether or not the CTMaximum was affected. The CTMaximum temperature of B. bidyanus decreased by 2.8, 3.8, and 0.3 degrees C on exposure to endosulfan, chlorpyrifos, and phenol, respectively. Similarly, in M. duboulayi, the CTMaximum was decreased by 4.1, 2.5, and 0 degrees C, while in H. klunzingeri it decreased by 3.1, 4.3, and 0.1degrees C, respectively, and in O. mykiss by 4.8, 5.9, and 0.7 degrees C, respectively. Exposure to sublethal test concentrations of endosulfan and chlorpyrifos caused significant ($p < or = 0.0001$) reductions in CTMaximum values for all fish species compared to that of unexposed fish. However,

exposure to phenol did not cause any significant (p > or = 0.05) change of CTMaximum temperatures.

15) Rakotondravelo, M.L., T.D. Anderson, R.E. Charlton, K.Y. Zhu, 2006, Sublethal Effects of Three Pesticides on Larval Survivorship, Growth, and Macromolecule Production in the Aquatic Midge, Chironomus tentans (Diptera: Chironomidae), Arch.Environ.Contam.Toxicol. 51(3):352-359.

Abstract: Effects of long-term exposure to each of three pesticides including atrazine, DDT, and chlorpyrifos on larval survivorship, growth, and macromolecule (total body protein and RNA) production were evaluated in the aquatic midge, Chironomus tentans, under laboratory conditions. Newly hatched larvae were exposed to atrazine at 30 and 150 microg/L, DDT at 0.01 and 0.05 microg/L, or chlorpyrifos at 0.02 and 0.10 microg/L throughout one life cycle. Larval survivorship was evaluated at 20 d and the end of the test, and larval growth at 20 d. Chlorpyrifos at 0.1 microg/L reduced the midge survivorship by 67% after 20-d exposure. However, neither atrazine nor DDT affected larval survivorship. The ash-free dry weight of midge larvae exposed to chlorpyrifos at 0.1 microg/L was 1.5-fold greater than that of the control whereas neither atrazine nor DDT showed a significant effect on the ash-free dry weight. In addition, exposures of midges to chlorpyrifos at 0.1 microg/L increased the adult emergence rate by 81% as compared to the control although the actual number of adults that emerged from chlorpyrifos-treated larvae was significantly decreased. Both the increased ash-free dry weight of larvae and increased adult emergence rate were likely caused by reduced competition for both food and space among the survivors due to increased larval mortality. Although neither total protein nor total RNA production was significantly affected in larvae exposed to each pesticide for 20 d, a significantly higher number of males over females (ratio = 4) emerged from midges exposed to DDT at 0.05 microg/L. Our study indicates that chronic exposure to low concentrations of chlorpyrifos and DDT results in significant mortality of midge larvae and alteration of the sex ratio of adult emergence, respectively. Because midges are important components of the food web, our results suggest that effects elicited directly or indirectly by long-term pesticide exposures may potentially disrupt both food chains and community structure in aquatic environments.

16) Rakotondravelo, M.L., T.D. Anderson, R.E. Charlton, K.Y. Zhu, 2006, Sublethal effects of three pesticides on activities of selected target and detoxification enzymes in the aquatic midge, Chironomus tentans (diptera: chironomidae). Arch.Environ.Contam.Toxicol. 51(3):360-366.

Abstract: Sublethal effects of three pesticides including atrazine (triazine herbicide), DDT (organochlorinated insecticide), and chlorpyrifos (organophosphate insecticide) on acetylcholinesterase (AChE), general esterase (GE), glutathione S-transferase (GST), and cytochrome P450 monooxygenase (P450) activities were evaluated in the aquatic midge Chironomus tentans. Exposures of midges to atrazine at 30 and 150 micrograms per liter (microg/L) for 20 d (i.e., from the first- to fourth-instar larvae) enhanced P450 O-

deethylation activity by 12.5- and 15.5-fold, respectively, but did not significantly change AChE, GST, and GE activities. Similar exposures to DDT at 0.01 and 0.05 microg/L did not significantly affect AChE, GE, and P450 activities; however, DDT at 0.05 microg/L enhanced GST activity toward the substrate 1-chloro-2, 4-dinitrobenzene by 33.6%. Exposures of midges to chlorpyrifos at 0.10 microg/L for 20 d reduced AChE activity by 59.8%, and GE activities toward the substrates alpha-naphthyl acetate and beta-naphthyl acetate by 30.7 and 48.8%, respectively. The reduced GE activities appear to be due to the inhibition of several esterases, particularly the one with a slow migration, by chlorpyrifos as demonstrated by non-denaturing polyacrylamide gel electrophoresis. Furthermore, exposure of midges to chlorpyrifos at 0.10 microg/L for 20 d enhanced the P450 O-deethylation activity by 3.3-fold although no significant effect was observed at 0.02 microg/L for the same enzyme. These results provide insights into the sublethal effects of these commonly detected pesticides in aquatic environments on important enzymes in aquatic midges.

17) Sparling, D.W., and G. Fellers, 2007, Comparative Toxicity of Chlorpyrifos, Diazinon, Malathion and Their Oxon Derivatives to Larval Rana boylii, Environ.Pollut. 147(3):535-539.

Abstract: Organophosphorus pesticides (OPs) are ubiquitous in the environment and are highly toxic to amphibians. They deactivate cholinesterase, resulting in neurological dysfunction. Most chemicals in this group require oxidative desulfuration to achieve their greatest cholinesterase-inhibiting potencies. Oxon derivatives are formed within liver cells but also by bacterial decay of parental pesticides. This study examines the toxicity of chlorpyrifos, malathion and diazinon and their oxons on the foothill yellow-legged frog (Rana boylii). R. boylii is exposed to agricultural pesticides in the California Central Valley. Median lethal concentrations of the parental forms during a 96 h exposure were 3.00 mg/L (24h) for chlorpyrifos, 2.14 mg/L for malathion and 7.49 mg/L for diazinon. Corresponding oxons were 10 to 100 times more toxic than their parental forms. We conclude that environmental concentrations of these pesticides can be harmful to R. boylii populations.

18) Sturm, A., T.S. Radau, T. Hahn, and R. Schulz, 2007, Inhibition of Rainbow Trout Acetylcholinesterase by Aqueous and Suspended Particle-Associated Organophosphorous Insecticides, Chemosphere 68(4):605-612.

Abstract: Spraydrift and edge-of-field runoff are important routes of pesticide entry into streams. Pesticide contamination originating from spraydrift usually resides in the water phase, while pesticides in contaminated runoff are to a large extent associated with suspended particles (SPs). The effects of two organophosphorous insecticides (OPs), chloropyrifos (CPF) and azinphos-methyl (AZP), on acetylcholinesterase (AChE) activity in rainbow trout were compared between two exposure scenarios, simulating spraydrift- and runoff-borne contamination events in the Lourens River (LR), Western Cape, South Africa. NOECs of brain AChE inhibition, determined after 1h of exposure followed by

24h of recovery, were 0.33microgl(-1) for aqueous CPF, 200mgkg(-1) for SP-associated CPF and 20mgkg(-1) for SP-associated AZP (at 0.5gl(-1) SP). The highest aqueous AZP concentration tested (3.3microgl(-1)) was without significant effects. Previously reported peak levels of aqueous CPF in the LR (approximately 0.2microgl(-1)) are close to its NOEC (this study), suggesting a significant toxicological risk to fish in the LR. By contrast, reported levels of SP-associated OPs in the LR are 20-200-fold lower than their NOECs (this study). In a comparative in situ study, trout were exposed for seven days at agricultural (LR2, LR3) and upstream reference (LR1) sites. No runoff occurred during the study. Brain AChE was significantly inhibited at LR3. However, OP levels at LR3 (CPF 0.01microgl(-1); AZP 0.14microgl(-1)) were minor compared to concentrations having effects in the laboratory (see above). Additionally, muscle AChE activity was significantly higher in caged trout from LR1 than in animals maintained in laboratory tanks.

19) Trimble, A.J., and M.J. Lydy, 2006, Effects of Triazine Herbicides on Organophosphate Insecticide Toxicity in Hyalella azteca, Arch.Environ.Contam.Toxicol. 51(1):29-34.

Abstract: The frequent use of pesticides in agricultural and commercial settings has led some researchers to devote their attention to studying the effects of mixtures of these compounds as they co-occur in the environment. Recent studies have demonstrated the potentiating effects of triazine herbicides, such as atrazine and its analogs, to the toxicity of a variety of organophosphate (OP) insecticides. One such OP insecticide, chlorpyrifos, has been the topic of much concern because of its prevalence in the environment. This study focused on examining the effects of 10 select triazine herbicides at concentrations of 1 mumole/L (approximately 200 mug/L) to chlorpyrifos with Hyalella azteca. The compounds selected include atrazine, three of its degradation products, and six other herbicide active ingredients. Toxicity tests were performed using a two-way analysis of variance matrix design with effect levels determined by way of probit analysis. Atrazine was found to have the greatest acutely lethal effect to H. azteca, followed by its closest degradation product, deethylatrazine. Two of the six atrazine analogs, simazine and cyanazine, also showed significant effects to the insecticide's toxicity. Synergistic ratios (SRs) were calculated to compare the effect magnitudes for each of the herbicides. The highest ratio obtained was with atrazine (SR = 1.42). A majority of the past studies involving mixtures of triazines and OPs have examined the potentiation effects of active-use triazine herbicides on Chironomus species. However, compared with the acute effects previously obtained for Chironomus species, H. azteca show a higher tolerance to the presence of the triazine herbicides, even at levels often considered as being at the high end of environmentally relevant concentrations. When coupled with past studies from our laboratory, this research helps to provide a better understanding of the toxic effects of herbicide-insecticide interactions.

20) Varo, I., F. Amat, J.C. Navarro, M. Barreda, E. Pitarch, and R. Serrano, 2006, Assessment of the Efficacy of Artemia sp (Crustacea) Cysts Chorion as Barrier to Chlorpyrifos (Organophosphorus Pesticide) Exposure. Effect on Hatching and Survival, Sci.Total Environ. 366(1):148-153.

Abstract: In order to reveal the efficacy of the Artemia cysts chorion as barrier to the organophosphorus pesticide chlorpyrifos, whole and decapsulated cysts have been exposed to 10 mg L(-1) chlorpyrifos in sea water during hydration and hatching phase, separately. The concentration of chlorpyrifos in capsulated and decapsulated cysts after exposure has been determined in order to elucidate the efficacy of chorion as protection to the embryo. The results obtained demonstrate the ability of the cysts chorion to obstruct the pass of chlorpyrifos molecules through this protection structure. Thus, the concentration of chlorpyrifos in exposed decapsulated cysts is higher than in exposed whole cysts. Moreover, after removing the chorion of exposed cysts, the concentration of chlorpyrifos in the embryo was lower than that of cysts exposed, what would demonstrate the retention of chlorpyrifos molecules by the shell. Hatching was not severely affected by exposure to the insecticide whereas survival at 44 h of the nauplii exposed to chlorpyrifos was significantly different from the controls. Survival of nauplii hatched from exposed decapsulated cysts was higher than that from those hatched from exposed whole cysts, probably because of the lower vitality of the latter, due to depletion of energy reserves during hatching.

21) Wacksman, M.N., J.D. Maul, and M.J. Lydy, 2006, Impact of Atrazine on Chlorpyrifos Toxicity in Four Aquatic Vertebrates, Arch.Environ.Contam.Toxicol. 51(4):681-689.

Abstract: Atrazine has been shown previously to potentiate chlorpyrifos toxicity in selected invertebrates. This study examined interactions of atrazine and chlorpyrifos in four aquatic vertebrates. Organisms were exposed to binary mixtures of atrazine and chlorpyrifos during toxicity bioassays. Inhibition of cholinesterase (ChE) enzyme activity and chlorpyrifos uptake kinetics were also examined with and without atrazine exposure. Atrazine alone did not affect organisms at concentrations up to 5000 microg/L; however, the presence of atrazine at 1000 microg/L did result in a significant increase in the acute toxicity of chlorpyrifos in Xenopus laevis. Mixed results were encountered with Pimephales promelas; some bioassays showed greater than additive toxicity, while others showed an additive response. No effect of atrazine on chlorpyrifos toxicity was observed for Lepomis macrochirus and Rana clamitans. Atrazine did not affect ChE activity or chlorpyrifos uptake rates, indicating that these toxicodynamic and toxicokinetic parameters may not be related to the mechanism of atrazine potentiation of chlorpyrifos toxicity. Based on the results of this study, it does not appear that a mixture toxicity of atrazine and chlorpyrifos at environmentally relevant concentrations presents a risk to the vertebrate organisms examined in this study.

Diazinon

1) Durmaz, H., Y. Sevgiler, and N. Uner, 2006, Tissue-Specific Antioxidative and Neurotoxic Responses to Diazinon in Oreochromis niloticus, Pestic.Biochem.Physiol. 84(3):215-226.

Abstract: Effects of diazinon, at different concentrations and exposure times, were investigated in freshwater fish, Cyprinus carpio, to elucidate the possible mode of action on lipid peroxidation together with the inhibitory effect of diazinon on acetylcholinesterase activity and changes in tissue protein levels. Cholinesterase inhibition is considered to be a specific biomarker of exposure to organophosphorus pesticides. Fish were exposed to 0.0036 microg/L, 0.018 microg/L, and 0.036 microg/L (sublethal) concentrations of diazinon for 5, 15, and 30 days, and biochemical measurements were carried out spectrophotometrically. Brain was chosen as an indicator tissue because it is a target system for the organophosphorus action. More than 20% decline in acetylcholinesterase activity relative to mean activity of the controls was observed in the diazinon-exposed groups. Protein content decreased significantly after 15 days of exposure to 0.018 microg/L and 0.036 microg/L diazinon and after 30 days of exposure to 0.036 microg/L. Malondialdehyde level declined markedly compared with the control levels. This study showed that prolonged exposures of C. carpio to diazinon had significant effects on brain acetylcholinesterase activity and that environmentally relevant concentrations of diazinon can significantly inhibit brain acetylcholinesterase activity. Altered protein content was probably due to the high energy demand under pesticide stress or inhibition of de novo enzyme synthesis. The decreased malondialdehyde content may reflect the possibility of better protection against oxidative stress.

2) Khoshbavar-Rostami, H.A., M. Soltani, and H.M.D. Hassan, 2006, Immune Response of Great Sturgeon (Huso huso) Subjected to Long-Term Exposure to Sublethal Concentration of the Organophosphate, Diazinon, Aquaculture 256(1-4):88-94.

Abstract: Lysozyme activity, chemiluminuscence (CL) response and immunocompetent cells population size were assessed in great sturgeon (Huso huso) weighing about 425 g following long-term exposure of fish to sublethal concentration of diazinon at 1.5 mg L-1 at 22 ±1 °C and acceptable water quality conditions. Samples were collected after 24 h and then every week interval for 9 weeks. Values of white blood cells (WBC) and lymphocyte in fish exposed to diazinon (group B) were significantly lower than unexposed group (group A) while, the level of neutrophils was higher ($P<0.05$). No significant differences were found in values of monocytes, eosinophils and immature neutrophils between these two groups ($P>0.05$). Also, values of WBC and neutrophils in intraperitoneally glucan injected fish (0.3 mg/kg body weight intraperitoneally as a single dose) plus diazinon bath (group D) were lower and higher than glucan received fish without diazinon bath (group C), respectively ($P<0.05$). Lymphocyte, monocyte and immature neutrophils values in group D were also higher than group C for the first 7, 14 and 28 days post-exposure, respectively and then they reduced to lower levels than group C during the rest of the experiment. Also, level of eosinophils in group D was lower than

group C (P>0.05). The level of lysozyme in liver and kidney tissues of group B was significantly and insignificantly higher (P< 0.05) than group A for 4 weeks post-treatment and then it reduced to lower levels until week 9 post-exposure. The levels of lysozyme in spleen and serum were also, significantly and insignificantly higher in group B than group A for the first 3-4 weeks post-exposure and, then they reduced to below the levels measured during the rest of the experiment (P>0.05). There was no significant difference in the lysozyme contents of liver, kidney, spleen and serum between groups C and D (P> 0.05). Mean spontaneous CL response in groups B and D was significantly lower than groups A and C throughout the experiment (P<0.05). Maximum peak of CL response was found in group C for 5 weeks post-exposure, while the minimum peak was found in group B throughout the experiment. Also, peak of CL response in group D was almost similar to that of group C up to 2 weeks post-exposure, but it significantly reduced to lower level than group C during the rest of the experiment.

3) Koprucu, S.S., K. Koprucu, M.S. Ural, U. Ispir, and M. Pala, 2006, Acute Toxicity of Organophosphorous Pesticide Diazinon and Its Effects on Behavior and Some Hematological Parameters of Fingerling European Catfish (Silurus glanis L.), Pestic.Biochem.Physiol. 86(2):99-105.

Abstract: Diazinon is commonly used for pest control in the agricultural fields surrounding freshwater reservoirs. So this study was conducted to determine the acute toxicity of this organophosphorous pesticide, contaminating aquatic ecosystems as a pollutant, and its effects on behavior, and some hematological parameters of fingerling European catfish, Silurus glanis. Diazinon was applied at concentrations of 1, 2, 4, 8, 16, and 64 mg L-1. The water temperature in the experimental units was kept at 16 ± 1°C. The number of dead fishes significantly increased in response to diazinon concentrations 2-64 mg L-1 (p<0.05). With increasing diazinon concentrations, the fishes exposed duration 1- 96 h significantly increased the number of dead fishes (p<0.05 for each cases). The 1, 24,48, 72, and 96 h LC50 values (with 95% confidence limits) of diazinon for fingerling European catfish were estimated as 14.597 (12.985-16.340), 12.487 (11.079-14.471), 8.932 (7.907-10.348), 6.326 (no data because of p>0.05), and 4.142 (no data because of p>0.05) mg L-1, respectively. Compared to the control specimens, fish after an acute exposure to diazinon was significantly lower erythrocyte, leukocyte, hemoglobin, hematocrit, MCV, MCH, and MCHC values (p<0.05). In addition, it was also showed a significantly negative correlation between these hematological parameters and exposure times of diazinon (p < 0.01).

4) Oruc, E.O., and D. Usta, 2007, Evaluation of Oxidative Stress Responses and Neurotoxicity Potential of Diazinon in Different Tissues of Cyprinus carpio, Environ.Toxicol.Pharmacol. 23(1):48-55. NOT AVAILABLE ONLINE.

Abstract: Toxicity of organophosphorus insecticides is mainly due to the inhibition of acetylcholinesterase, but, oxidative stress may be involved in the toxicity of this pesticides. Therefore, it was investigated whether diazinon, a commonly used organophosphate, may

induce oxidative stress and cholinesterase inhibition in different tissues of *Cyprinus carpio*. Sublethal concentrations of diazinon (0.0036, 0.018 and 0.036 ppb) were administered to *C. carpio* L. for 5, 15 and 30 days. The study was made by measuring biochemical stress responses of *C. carpio* L. spectrophotometrically taking into account acetylcholinesterase (AChE), Na^+K^+-adenosine triphosphatase (Na^+K^+-ATPase) and other antioxidant enzyme activities, as well as malondialdehyde and protein contents in gill, muscle and kidney tissues of the fish. Results of the study suggest that AChE (in gill and muscle tissues) and Na^+K^+-ATPase (in muscle and kidney tissues) activities decreased; that antioxidant enzymes, in particular superoxide dismutase (SOD), increased in gill, kidney and muscle tissues. We also observed the existence of a protective function of antioxidant enzymes against lipid peroxidation in muscle tissue. The changes in MDA content varied between increases and decreases in kidney tissue. In gill tissue, however, lipid peroxidation could not be prevented despite induction of SOD and glutathione peroxidase activities. We could see that the protein content decreased only in gill tissue as diazinon dosage was gradually increased until the 15th day of the experiment. During the period between 15th and the 30th days, the protein level in the fish was observed to have reached to that of the control group. This change in protein level can be attributed to adjustment of the fish to its new environmental conditions. Considering most of the parameters in tissues, it can be stated that diazinon exerted its effect at low concentration and during a long period of time, and its toxicity increased dose dependently. This study reveals that *C. carpio* developed tissue-specific adaptive response to neutralize the oxidative stress following pesticide exposure depending on different antioxidant levels in tissues and that SOD can be used as a biomarker in determining diazinon toxicity due to its early response at even low concentration levels

5) Oruc, E.O., N. Uner, Y. Sevgiler, D. Usta, and H. Durmaz, 2006, Sublethal Effects of Organophosphate Diazinon on the Brain of Cyprinus carpio, Drug Chem.Toxicol. 1(29):57-67.

Abstract: Effects of diazinon, at different concentrations and exposure times, were investigated in freshwater fish, Cyprinus carpio, to elucidate the possible mode of action on lipid peroxidation together with the inhibitory effect of diazinon on acetylcholinesterase activity and changes in tissue protein levels. Cholinesterase inhibition is considered to be a specific biomarker of exposure to organophosphorus pesticides. Fish were exposed to 0.0036 microg/L, 0.018 microg/L, and 0.036 microg/L (sublethal) concentrations of diazinon for 5, 15, and 30 days, and biochemical measurements were carried out spectrophotometrically. Brain was chosen as an indicator tissue because it is a target system for the organophosphorus action. More than 20% decline in acetylcholinesterase activity relative to mean activity of the controls was observed in the diazinon-exposed groups. Protein content decreased significantly after 15 days of exposure to 0.018 microg/L and 0.036 microg/L diazinon and after 30 days of exposure to 0.036 microg/L. Malondialdehyde level declined markedly compared with the control levels. This study showed that prolonged exposures of C. carpio to diazinon had significant effects on brain acetylcholinesterase activity and that environmentally relevant concentrations of diazinon

can significantly inhibit brain acetylcholinesterase activity. Altered protein content was probably due to the high energy demand under pesticide stress or inhibition of de novo enzyme synthesis. The decreased malondialdehyde content may reflect the possibility of better protection against oxidative stress.

6) Schiff K, Sutula M. 2004, Organophosphorus pesticides in storm-water runoff from southern California (USA). Environ Toxicol Chem. 23(8):1815-21.

Abstract: Large quantities of the organophosphorus (OP) pesticides diazinon and chlorpyrifos are applied to California (USA) watersheds every year, but few data are available on the sources of OP pesticides in urban watersheds. The goal of this study was to characterize diazinon and chlorpyrifos concentrations from different land uses indicative of source categories in urban southern California watersheds. This characterization included analysis of 128 runoff samples from eight different land uses over five storm events. Diazinon was consistently detected (93% of samples) during this study, whereas chlorpyrifos was not consistently detected (12% of samples). The mixed agricultural land use had the highest flow weighted mean (FWM) concentration of diazinon (4076 ng/L), which exceeded the next-highest land-use categories (commercial and residential) by one to two orders of magnitude (324-99 ng/L, respectively). Open space had the lowest concentration of diazinon (<20 ng/L). Concentrations of diazinon at replicate land-use sites and during replicate storm events at the same site were highly variable. The difference in diazinon FWM concentrations among replicate sites ranged from 1.5-fold to 45-fold. The difference in diazinon FWM concentrations among storms at the same site ranged from 1.25-fold to 30-fold. Part of this variability is a response to the temporal patterns observed within a storm event. The majority of land-use site-events had peak concentrations before peak flow indicating a first-flush effect, but this was not always a predictable temporal trend. The first-flush effect was rarely evident in terms of mass loadings because flows can range orders of magnitude during a single event in highly impervious urban watersheds. Flow variability thus overwhelms the variability in diazinon concentrations attributable to the first-flush effect.

7) Sparling, D.W., and G. Fellers, 2007, Comparative Toxicity of Chlorpyrifos, Diazinon, Malathion and Their Oxon Derivatives to Larval Rana boylii, Environ.Pollut. 147(3):535-539.

Abstract: Organophosphorus pesticides (OPs) are ubiquitous in the environment and are highly toxic to amphibians. They deactivate cholinesterase, resulting in neurological dysfunction. Most chemicals in this group require oxidative desulfuration to achieve their greatest cholinesterase-inhibiting potencies. Oxon derivatives are formed within liver cells but also by bacterial decay of parental pesticides. This study examines the toxicity of chlorpyrifos, malathion and diazinon and their oxons on the foothill yellow-legged frog (Rana boylii). R. boylii is exposed to agricultural pesticides in the California Central Valley. Median lethal concentrations of the parental forms during a 96 h exposure were 3.00 mg/L (24h) for chlorpyrifos, 2.14 mg/L for malathion and 7.49 mg/L for diazinon.

Corresponding oxons were 10 to 100 times more toxic than their parental forms. We conclude that environmental concentrations of these pesticides can be harmful to R. boylii populations.

8) Van Cong, N., N.T. Phuong, and M. Bayley, 2006, Sensitivity of Brain Cholinesterase Activity to Diazinon (Basudin 50EC) and Fenobucarb (Bassa 50EC) Insecticides in the Air-Breathing Fish Channa striata (Bloch, 1793), Environ. Toxicol. Chem. 25(5):1418-1425.

Abstract: With the expansion of agricultural areas within the Mekong River Delta in Vietnam, a concurrent, dramatic increase has occurred in agrochemical usage. To date, little consideration has been given to the negative impacts of this agricultural activity on the aquatic resources of the region. Both acute toxicity and subacute effects on brain cholinesterase (ChE) of two of the most commonly used insecticides, diazinon and fenobucarb, on adult native snakehead (Channa striata) were evaluated in a static, nonrenewable system, the environmental parameters of which, such as dissolved oxygen, water temperature, and pH, fluctuated similarly to field conditions. Four levels of insecticides, from 0.008 to 0.52 mg/L (for diazinon) and from 0.11 to 9.35 mg/L (for fenobucarb), were tested to assess the effects on the brain ChE activity of the snakehead up to 30 and 10 d for diazinon and fenobucarb, respectively. Diazinon was highly toxic to this fish species, with a 96-h median lethal concentration (LC50) of only 0.79 mg/L, and it also caused long-term ChE inhibition, with activity still significantly inhibited by 30% after 30 d for the three highest concentrations. Fenobucarb was less toxic to this species, with a 96-h LC50 of 11.4 mg/L. Fenobucarb caused more rapid ChE inhibition but also rapid recovery. The results of the present study indicate an urgent need to regulate the usage of these pesticides in the Mekong River Delta.

9) Viant, M.R., C.A. Pincetich, and R.S. Tjeerdema, 2006, Metabolic Effects of Dinoseb, Diazinon and Esfenvalerate in Eyed Eggs and Alevins of Chinook Salmon (Oncorhynchus tshawytscha) Determined by 1H NMR Metabolomics, Aquat.Toxicol. 77(4):359-371.

Abstract: Pesticide pulses in the Sacramento River, California, originate from storm-water discharges and non-point source aquatic pollution that can last from a few days to weeks. The Sacramento River and its tributaries have historically supported the majority of California's Chinook salmon (Oncorhynchus tshawytscha) spawning grounds. Three pesticides currently used in the Sacramento Valley – dinoseb, diazinon, and esfenvalerate – were chosen to model the exposure of salmon embryos to storm-water discharges. Static-renewal (96 h) exposures to eyed eggs and alevins resulted in both toxicity and significant changes in metabolism assessed in whole-embryo extracts by (1)H nuclear magnetic resonance (NMR) spectroscopy based metabolomics and HPLC with UV detection (HPLC-UV). The 96-h LC(50) values of eyed eggs and alevins exposed to dinoseb were 335 and 70.6 ppb, respectively, and the corresponding values for diazinon were 545 and 29.5 ppm for eyed eggs and alevins, respectively. The 96-h LC(50) of eyed eggs exposed to esfenvalerate could not be determined due to lack of mortality at the highest exposure

concentration, but in alevins was 16.7 ppb. All esfenvalerate exposed alevins developed some degree of lordosis or myoskeletal abnormality and did not respond to stimulus or exhibit normal swimming behavior. ATP concentrations measured by HPLC-UV decreased significantly in eyed eggs due to 250 ppb dinoseb and 10 and 100 ppb esfenvalerate ($p < 0.05$). Phosphocreatine, as measured by HPLC-UV, decreased significantly in eyed eggs due to 250 ppb dinoseb, 10 and 100 ppb esfenvalerate, and 100 ppm diazinon ($p < 0.05$). Principal components analyses of (1)H NMR metabolite fingerprints of eyed egg and alevin extracts revealed both dose-dependent and mechanism of action-specific metabolic effects induced by the pesticides. Furthermore, NMR based metabolomics proved to be more sensitive than HPLC-UV in identifying significant changes in sublethal metabolism of pesticide exposed alevins. In conclusion, we have demonstrated several benefits of a metabolomics approach for chemical risk assessment, when used in conjunction with a fish embryo assay, and have identified significant metabolic perturbations to the early life stages of Chinook salmon by currently used pesticides.

Malathion

1) Sparling, D.W., and G. Fellers, 2007, Comparative Toxicity of Chlorpyrifos, Diazinon, Malathion and Their Oxon Derivatives to Larval Rana boylii, Environ.Pollut. 147(3):535-539.

Abstract: Organophosphorus pesticides (OPs) are ubiquitous in the environment and are highly toxic to amphibians. They deactivate cholinesterase, resulting in neurological dysfunction. Most chemicals in this group require oxidative desulfuration to achieve their greatest cholinesterase-inhibiting potencies. Oxon derivatives are formed within liver cells but also by bacterial decay of parental pesticides. This study examines the toxicity of chlorpyrifos, malathion and diazinon and their oxons on the foothill yellow-legged frog (Rana boylii). R. boylii is exposed to agricultural pesticides in the California Central Valley. Median lethal concentrations of the parental forms during a 96 h exposure were 3.00 mg/L (24h) for chlorpyrifos, 2.14 mg/L for malathion and 7.49 mg/L for diazinon. Corresponding oxons were 10 to 100 times more toxic than their parental forms. We conclude that environmental concentrations of these pesticides can be harmful to R. boylii populations.

2) Yeh, H.J., and C.Y. Chen, 2006, Toxicity Assessment of Pesticides to Pseudokirchneriella subcapitata Under Air-Tight Test Environment, J.Hazard.Mater.A 131(1-3):6-12.

Abstract: This paper presents the toxicity data of seven pesticides including atrazine, parathion, dichlorvos, malathion, fenthion, 2-methyl-4-chlorophenoxyacetic acid, and pentachlorophenol on Pseudokirchneriella subcapitata based on a new algal toxicity testing technique conducted under air-tight environment. The dissolved oxygen production and the cell density were adopted as the response endpoints. Median effective concentrations (EC50) range from 0.0035 to 3.40 mg/L (DO production) and from 0.0067 to 3.12 mg/L

(cell density). No-observed-effect concentration (NOEC) was determined using the Dunnett's test. NOEC values are with in the range of 0.001-1.20 mg/L. In general, the two test endpoints revealed similar sensitivities. From comparisons of literature data also based on Pseudokirchneriella subcapitata, it is clear that conventional batch tests tend to underestimate the toxicity of pesticides due to their open test environment. Closed-system tests, i.e., microplate test, respirometer test, and our BOD-bottle test, generally provide better assessment to the effects of pesticides. Data based on our test method reveals much higher toxicity (3-100 times) than that from the conventional batch tests. Furthermore, for organophosphorus insecticides, results from the present study show that Pseudokirchneriella subcapitata is less sensitive than Daphnia magna and rainbow trout, but is more susceptible than fathead minnow. The closed-system test applied in this study provides more adequate assessment for the toxicity of pesticides than the conventional batch tests.