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Toxicity of Chlorpyrifos Adsorbed on Humic Colloids to Larval Walleye (*Stizostedion vitreum*)

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Abstract. After application, organophosphorus insecticides (OPs) are often strongly adsorbed to soil constituents. Because of their relatively low water solubility, OPs may be transferred from field to stream adsorbed on suspended solids. However, we are not aware of research done to evaluate the bioavailability (i.e., toxicity) of OPs transported on suspended solids to fish. We conducted 48-h static toxicity tests to determine the toxicity of chlorpyrifos in aqueous solution and adsorbed on calcium-saturated humic acid (HA) to three larval stages of walleye (*Stizostedion vitreum*). Three concentrations of chlorpyrifos adsorbed on HA, a HA control, and a chlorpyrifos-only treatment were tested. Fish that survived the 48-h static toxicity tests were analyzed to determine total cholinesterase (ChE) activity. In general, survival of all larval stages of walleye exposed to chlorpyrifos–HA complexes was less than that of walleye exposed to HA controls and the chlorpyrifos-only treatment, which were not toxic to walleye. Cholinesterase inhibition of larval walleye exposed to chlorpyrifos–HA complexes was similar to the ChE inhibition observed in larval walleye exposed to chlorpyrifos in the aqueous phase. These laboratory experiments indicate potential toxicity of chlorpyrifos–soil complexes to larval fish.

Since the 1970s, the use of organochlorine insecticides in the United States has been banned or greatly curtailed due to problems from environmental persistence and biomagnification (Pait *et al.* 1992; Richmonds and Dutta 1992). As a result, the use of organophosphorus insecticides (OPs) increased rapidly and by 1989, OPs were the most widely used group of insecticides in the world, representing nearly 40% of the insecticide market (Racke 1993). In 1993, in the United States, the most widely used insecticide was chlorpyrifos; more than 3 million kg were applied to field crops (ERS 1994). In addition to their widespread use, OPs have a high acute toxicity to aquatic organisms; for example, the 96-h LC50 for bluegill (*Lepomis macrochirus*) exposed to chlorpyrifos is 2.4 μg/L (Mayer and Ellersieck 1986).

Organophosphorus insecticides have low water solubilities (e.g., chlorpyrifos has a solubility of 1.39 mg/L at 25°C), high soil adsorption coefficients (Kd; chlorpyrifos has Kd values ranging from 13.4 to 1,862 mL/g depending on the soil type) (Harris 1972; Racke 1993), and once adsorbed are difficult to desorb from soil constituents. Thus, the greatest likelihood of OP exposure to aquatic life may occur when OPs are transported off-site adsorbed to organic and inorganic soil constituents. Although research has been done to evaluate the toxicity of sediment-associated chlorpyrifos to a marine copepod (Green *et al.* 1996), we are not aware of research on biological availability of OPs bound on suspended soil constituents to fish. However, Misitano *et al.* (1994) demonstrated that polynuclear aromatic hydrocarbons and polychlorinated biphenyls sorbed to sediment were transferred to larval fish. This work demonstrates the potential toxicity to aquatic organisms of hydrophobic compounds bound to suspended solids.

The most likely route of exposure of fish to OPs adsorbed on suspended solids involves contact between suspended solids and gill tissue, a metabolically active epithelium. Consequently, the bioavailability of suspended solid-adsorbed OPs depends on the strength of the suspended solid-OP bonding, the strength of interaction between OPs and gill tissue, and the extent of exposure of gills to suspended solids containing OPs. The objective of this study was to evaluate the sublethal effect as measured by cholinesterase inhibition and acute toxicity of chlorpyrifos adsorbed on calcium-saturated humic acid (HA) to larval walleye (*Stizostedion vitreum*).

Materials and Methods

Test Organisms and Culture Conditions

Eyed walleye eggs were obtained from the Spirit Lake Fish Hatchery, Spirit Lake, Iowa, on April 21, 1999, and incubated at 13.0°C in standard hatching jars for 5 days before hatching began. To maintain uniformity of age, only larvae that hatched within a 24-h interval were

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used in this study. Mean length ± SE of 20 larvae at hatching was 7.4 ± 0.05 mm. At 3 days posthatch, larvae were stocked at a density of 20 larvae/L in a 150-L tank (3,000 larvae/tank) for rearing to the stages needed for these experiments. Walleye were raised at 17.2 ± 0.2°C as described by Summerfelt (1996).

A sample of five walleye was netted every day from the culture tank. Walleye were then euthanized with 300 mg/L Finquel® (tricaine methane sulfonate), measured, and observed microscopically for the presence of the yolk sac and oil globule. Observations of the day when the yolk sac and oil globule disappeared were used to describe the larval stage. The three larval stages of walleye, as described by Li and Mathias (1982), are prolarval (yolk sac present: 1–5 days posthatch), postlarval I (yolk sac absent and oil globule present: 6–14 days posthatch), and postlarval II (oil globule absent: 15–21 days posthatch).

Water Quality

Temperature, dissolved oxygen (DO), pH, hardness, alkalinity, and total ammonia-nitrogen (TAN) were measured at the beginning, middle, and end of each 48-h toxicity test. Temperature was measured with a glass thermometer, and DO was measured with an oxygen-sensitive membrane electrode (polarographic) with a Yellow Springs model-56 meter (Yellow Springs Instrument Company, OH, USA). Total ammonia-nitrogen (NH_3-N) was measured using the Nesslerization method (APHA 1998) with a DR/3000 spectrophotometer (Hach Company, Loveland, CO, USA). Hardness was measured with the Man Ver two-burette titration method (Hach Company, Loveland, CO, USA) and total alkalinity was measured by titration with 0.02N H_2SO_4 and 0.01N NaOH (APHA 1998). The pH was measured with a model 8102 standard combination electrode and a model 420A pH meter (Orion Research, Cambridge, MA, USA). No differences in any water quality parameters were found among treatments and all water quality parameters were within the standards recommended to protect health of fish (Wedemeyer 1996).

Chemicals

Reagent-grade chlorpyrifos (99.6%) was purchased from Chem Services (West Chester, PA, USA). All chemicals required for cholinesterase (ChE) analysis were purchased from Sigma Chemical (St. Louis, MO, USA), and all chemicals for water quality analysis were purchased from HACH Company (Loveland, CO, USA). The humic acid was obtained from Aldrich Chemical Company (Milwaukee, WI, USA).

Preparation of Chlorpyrifos–HA Complexes

Humic acid was washed with 1.0 M $CaCl_2$ four times to prepare Ca-saturated humic acid, dialyzed using Spectra/Por® 3500 MWCO molecular porous membrane tubing against Milli-Q water until no Cl^- was detected by $AgNO_3$ and then freeze dried. Stock ethanol solutions containing 34, 109, and 340 μg/mL chlorpyrifos were prepared. One mL of the stock solution was shaken for 24 h with 19 mL of 0.01 M $CaCl_2$ containing 2 g of previously prepared HA. After 24 h, chlorpyrifos–HA complexes were added to test chambers containing 3.5 L of reference water (DO, 8.5–9.8 mg/L; pH 7.2–7.5; hardness, 148–164 mg/L as $CaCO_3$; alkalinity, 39–45 mg/L $CaCO_3$).

Acute Toxicity Assays

Forty-eight-hour static acute toxicity tests were conducted on three stages of larval walleye. Food was withheld 24 h preceding and during the 48-h exposure. A test consisted of three concentrations of chlorpyrifos adsorbed on 2 g of HA, a HA control, and a chlorpyrifos-only treatment. Each treatment was replicated three times within a test. The concentrations tested were HA control (2 g HA; no chlorpyrifos), low chlorpyrifos–HA (34 μg chlorpyrifos/3.5 L; 2 g HA), moderate chlorpyrifos–HA (109 μg chlorpyrifos/3.5 L; 2 g HA), high chlorpyrifos–HA (340 μg chlorpyrifos/3.5 L; 2 g HA), and chlorpyrifos (5–8 μg/L with no HA).

Exposures were conducted as follows: 50 prolarvae, 50 postlarvae I, and 25 postlarvae II walleye were randomly assigned to 3.5-L test solution in 4-L test chambers for each replicate. The glass test chambers were similar to those described by Schmidt-Dallmier *et al.* (1992), except 1.0-mm mesh screen was used to cover the notches cut at the top and bottom edges of the glass funnel to prevent larval walleye from passing through the notches. Suspension of HA was maintained with a propeller-tipped stirring rod driven by an electric motor with a rheostat. The revolution rates of all stir rods were synchronized at 1500 rpm with a stroboscope before additions of HA to the chambers. Test chambers were incubated in 15.5–18.0°C water baths (depending on the larval stage) throughout the 48-h exposure to maintain a constant temperature. At the end of the 48-h exposure, all surviving fish were euthanized with Finquel, placed in cryovials, and frozen in liquid nitrogen for later ChE analysis.

Concentrations of chlorpyrifos were measured from each chlorpyrifos–HA treatment midway through and at the end of each 48-h static toxicity test and at the beginning and end of each 48-h static toxicity test in chlorpyrifos-only treatments. Water samples were collected (10 mL) and placed in acetone-cleaned, amber glass jars. After collection, jars were stored at 4°C until analysis.

Concentrations of chlorpyrifos were measured using solid phase microextraction and gas chromatography (GC) (Wu 2000). A solid phase microextraction fiber coated with 85-μm polyacrylate (Supelco, Bellefonte, PA, USA) was immersed into 10-mL water samples that were stirred at a constant rate for 30 min. The solid phase microextraction fiber was then inserted directly into the GC injector port of a Hewlett Packard 5890 series II gas chromatograph (Wilmington, DE, USA) equipped with a flame ionization detector for desorption and analysis of chlorpyrifos. A split/splitless GC injection port maintained at 220°C, and a 30 m by 0.25 mm ID DB-1701 fused silica capillary column with a 0.25 μm stationary film (J and W Scientific, Folsom, CA, USA) was used. The GC oven was ramped from an initial temperature of 50°C to a final temperature of 260°C at a rate of 5°C/min. The final temperature was held for 10 min. Analyte desorption from the fiber and purge-off time was 5 min. The carrier gas was helium with a head pressure set to 10 psi, and the detector temperature was maintained at 260°C. A linear calibration ($r > 0.999$) was achieved for chlorpyrifos over the concentration range of the study (0–1,000 μg/L).

Cholinesterase Inhibition and Analysis

Fifteen individual prolarvae (three prolarvae were pooled for each analysis) and five postlarvae I and postlarvae II walleye from each test chamber that survived the 48-h static toxicity tests were analyzed to determine total ChE activity. Cholinesterase activity was analyzed in homogenates of whole individual larval fish. A colorimetric method for analyzing whole body ChE activity, modified for use on a THERMOmax microplate reader and SOFTmax software (Molecular Devices Corporation, Sunnyvale, CA, USA), was used to monitor the rate of formation of 5-thio-2-nitrobenzoate, a yellow-colored anion. Hydrolysis of acetylthiocholine (AThCh) by ChE results in an acetate ion

and a negatively charged thiocholine complex that reacts with 5,5-dithiobis-2-nitrobenzoic acid to form 5-thio-2-*p*-nitrobenzoate (Ellman *et al.* 1961; Hill and Fleming 1982; Gard and Hooper 1993). The microplate reader was set in the kinetic mode to monitor increases in absorbance at 405 nm, as previously described by Gard and Hooper (1993) and Beauvais *et al.* (2000). The optimal substrate concentration, 0.001 M AThCh, for larval walleye was determined prior to analysis with non-test samples. The V_{max} and dilution factors were used to calculate ChE activities, reported as μM AThCh hydrolyzed/min/g of tissue (μM/min/g).

All tissue samples analyzed for ChE activity were run in triplicate. If the coefficient of variation among the triplicates was greater than 10%, samples were rerun. Because a commercial ChE standard for walleye tissue was not available, a check standard was used. The check standard was made by pooling larval walleye diluted 100-fold in pH 7.4 Tris buffer. The pooled tissue was homogenized and divided into 1-mL aliquots in 2-mL cryovials and placed in liquid nitrogen. These aliquots were run as check standards in triplicate along with each plate of treated samples throughout the study to verify accuracy of the test. If the coefficient of variation was greater than 10%, all samples were rerun.

Statistical Methods

Differences in treatment effects on survival and/or ChE activity and water quality parameters were assessed by analysis of variance using Statview® software (SAS, Inc., Cary, NC, USA). When the F-value for the overall test was significant ($p < 0.05$), Fisher's least significant difference test was used to determine significance among treatments. Survival data were transformed to a normal distribution before analysis using the following formula: transformed survival = arcsine (survival proportion)$^{1/2}$ (Zar 1984).

Results

Prolarvae

Survival of 3-day-old prolarval walleye (mean length ± SE = 7.3 ± 0.04 mm) was significantly lower for fish exposed to the high chlorpyrifos–HA treatment compared with all other treatments ($p < 0.05$; Table 1). Also, ChE activity of prolarvae that survived the 48-h exposure decreased with increasing concentrations of chlorpyrifos. Prolarvae in the HA-control treatment had significantly higher ChE activity than prolarvae in any treatment group, but ChE activity in the high chlorpyrifos–HA treatment and the chlorpyrifos-only treatment were similar (Table 1).

Chlorpyrifos (2–4 μg/L) was detected in the aqueous phase after 24 and 48 h in the three HA–chlorpyrifos treatment groups (Table 1). The overall means ± SE for DO and pH were 9.2 ± 0.03 mg/L and 7.6 ± 0.06, respectively; hardness was 162 ± 0.5 mg/L; alkalinity was 40 ± 0.4 mg/L; and the temperature maintained by the water bath was 15.9 ± 0.01°C. Means of water quality variables among individual test chambers did not differ significantly.

Postlarvae I

No 9-day-old postlarvae I walleye (mean length ± SE = 9.4 ± 0.09 mm) survived the 48-h exposure to the high chlorpyrifos–HA treatment and only 2% of fish survived exposure to the moderate chlorpyrifos–HA treatment (Table 2). Survival was not significantly different among the HA-control, low chlorpyrifos–HA, and chlorpyrifos-only treatments. Cholinesterase activity was significantly higher in the HA control than in the treatments containing chlorpyrifos (Table 2).

Low concentrations of chlorpyrifos (2–4 μg/L) were detected in the aqueous phase after a 24-h equilibration period (Table 2). The overall means ± SE for DO and pH were 8.4 ± 0.05 mg/L and 7.4 ± 0.03, respectively; hardness was 168 ± 1.0 mg/L; alkalinity was 48 ± 1.9 mg/L; and the temperature maintained by the water bath was 17.7 ± 0.02°C. Water quality variables among individual test chambers did not differ significantly.

Postlarvae II

No 16-day-old postlarvae II walleye (mean length ± SE = 12.8 ± 0.21 mm) survived the 48-h exposure to the high chlorpyrifos–HA treatment, and survival was significantly lower for fish exposed to the low and moderate chlorpyrifos–HA treatments compared with the HA-control and chlorpyrifos-only treatments (Table 3). Cholinesterase activity was significantly higher in the HA control treatment compared with fish in treatments containing chlorpyrifos (Table 3).

Low concentrations of chlorpyrifos (3–5 μg/L) were detected in the aqueous phase after a 24-h equilibration period (Table 3). The overall means ± SE for DO and pH were 7.7 ± 0.16 mg/L and 7.4 ± 0.04, respectively; hardness was 177 ± 2.4 mg/L; alkalinity was 49 ± 2.1 mg/L; and the temperature maintained by the water bath was 17.8 ± 0.04°C. Water quality variables among individual test chambers did not differ significantly.

Discussion

The concentrations of chlorpyrifos in the chlorpyrifos-only treatments at the end of the 48-h experiments for each life stage were below detection limits (<1.0 μg/L; Tables 1, 2, and 3). This most likely occurred because fish are able to rapidly absorb chlorpyrifos from the exposure water and then rapidly metabolize chlorpyrifos (Barron *et al.* 1993; Deneer 1994). For example, concentrations of the chlorpyrifos parent compound decreased from a concentration of 12 μg/L to undetectable concentrations (<0.5 μg/L) at 24 h in water containing channel catfish (*Ictaluras punctatus*) (Barron *et al.* 1993).

Wu (2000) found that chlorpyrifos was nearly completely adsorbed on Ca-humate and did not desorb back into an aqueous solution. In this study, although chlorpyrifos was strongly adsorbed to HA, low concentrations of chlorpyrifos (2–5 μg/L) were detected in the aqueous phase for each larval stage experiment. However, concentrations of chlorpyrifos in the aqueous phase were not high enough to cause acute toxicity to larval walleye. Phillips *et al.* (2002) found static 48-h LC50s for chlorpyrifos to be 225–316, 24–29, and 12–13 μg/L for prolarvae, postlarvae I, and postlarvae II walleye, respectively.

Compared with the HA control, chlorpyrifos adsorbed on HA reduced survival of all stages of larval walleye. These data

Table 1. Survival and ChE activity (μM acetylthiocholine hydrolyzed/min/g tissue) of prolarvae (3-day-old) walleye after a 48-h exposure to chlorpyrifos–HA complexes

Total chlorpyrifos added (μg)[1]	Total HA added (g)	Chlorpyrifos in solution (μg/L)[2]		Survival (% ± SE)	ChE activity
		Midway	End		
0	2	<1	<1	87 ± 2.9[a]	5.24 ± 0.11[a]
34	2	2	2	88 ± 8.3[a]	2.78 ± 0.15[b]
109	2	3	2	91 ± 2.7[a]	2.41 ± 0.18[b]
340	2	4	4	45 ± 19.0[b]	1.77 ± 0.20[c]
28[3]	0	5	<1	92 ± 6.0[a]	1.93 ± 0.07[c]
P-value of ANOVA				0.031	<0.001
48-h LC50 (μg/L)[4]	225–316				

Survival and ChE activity differences for treatment groups followed by the same letter are not statistically significant ($p = 0.05$).
[1] Total amount of chlorpyrifos added to each test chamber at the beginning of the experiment.
[2] Concentration of chlorpyrifos was measured by GC, limit of detection is 1.0 μg/L.
[3] Water samples for analysis of chlorpyrifos were collected at the beginning and end of the 48-h toxicity test.
[4] 48-h LC50 for prolarvae walleye (Phillips *et al.* 2002).

Table 2. Survival and ChE activity (μM acetylthiocholine hydrolyzed/min/g tissue) of postlarvae I (9-day-old) walleye after a 48-h exposure to chlorpyrifos–HA complexes

Total chlorpyrifos added (μg)[1]	Total HA added (g)	Chlorpyrifos in solution (μg/L)[2]		Survival (% ± SE)	ChE activity
		Midway	End		
0	2	<1	<1	67 ± 8.4[a]	8.22 ± 0.85[a]
34	2	2	1	49 ± 9.8[a]	2.73 ± 0.32[b]
109	2	2	2	2 ± 2.0[b]	2.25
340	2	4	3	0	NA
28[3]	0	8	<1	58 ± 13.0[a]	2.40 ± 0.29[b]
P-value of ANOVA				<0.001	<0.001
48-h LC50 (μg/L)[4]	24–29				

Survival and ChE activity differences for treatment groups followed by the same letter are not statistically significant ($p = 0.05$).
[1] Total amount of chlorpyrifos added to each test chamber at the beginning of the experiment.
[2] Concentration of chlorpyrifos was measured by GC.
[3] Water samples for analysis of chlorpyrifos were collected at the beginning and end of the 48-h toxicity test.
[4] 48-h LC50 for postlarvae I walleye (Phillips *et al.* 2002).

suggest that chlorpyrifos may be biologically active in the aquatic environment when it is adsorbed on the organic fraction of suspended solids. Similarly, Misitano *et al.* (1994) found that radiolabeled polynuclear aromatic hydrocarbons and polychlorinated biphenyls sorbed to sediment were accumulated by larval surf smelt (*Hypomesus pretiosus*), demonstrating a mechanism for transfer of chemicals directly from sediment to fish.

Although survival was significantly lower for prolarvae exposed to the high chlorpyrifos–HA treatment, ChE activity of these prolarvae was not significantly different from the chlorpyrifos treatment. These results indicate that exposure to chlorpyrifos adsorbed on HA contributed to the mortality of larval walleye. However, ChE activity was only inhibited 66% in prolarvae exposed to high chlorpyrifos–HA, whereas 80–90% inhibition is usually required for death to occur (Weiss 1961; Coppage 1972; Cole 1995). Also, ChE activity in postlarvae I was inhibited 72% in the moderate chlorpyrifos–HA treatment and 71% in the chlorpyrifos treatments, but survival was only 2% in the moderate chlorpyrifos–HA treatment compared to 58% in the chlorpyrifos treatment. These results indicate that the chlorpyrifos–HA complexes were toxic to larval walleye.

The decreased ChE activity in postlarvae II walleye exposed to the moderate chlorpyrifos–HA treatment compared with the chlorpyrifos treatment may be explained by increased exposure from chlorpyrifos being transferred directly from the HA to the fish. This explanation is possible because similar concentrations of chlorpyrifos were in the aqueous phase of both treatments. In addition, because of its low water solubility, it is reasonable to assume that chlorpyrifos is lipophilic and diffuses across the gill membrane after gill development.

Overall, the postlarval II stage was found to be the most sensitive life stage to chlorpyrifos–HA complexes, followed by the postlarval I and prolarval life stages. Similar results have been found in larval walleye exposed to hydrogen peroxide and elevated pH. Bergerhouse (1992) found that less than 10% of prolarval walleye died when exposed to pH 10.0, but over 50% of postlarvae II died when exposed to the same pH. Clayton and Summerfelt (1996) found that only 2% of postlarvae I walleye, but 80% of prolarvae, survived a 1-h exposure to 100 mL/L hydrogen peroxide.

Although survival rates of 67% and 56% for postlarvae I and postlarvae II, respectively, exposed to the HA control (Tables

Table 3. Survival and ChE activity (μM acetylthiocholine hydrolyzed/min/g tissue) of postlarvae II (16-day-old) walleye after a 48-h exposure to chlorpyrifos–HA complexes

Total chlorpyrifos added (μg)[1]	Total HA added (g)	Chlorpyrifos in solution (μg/L)[2]		Survival (% ± SE)	ChE activity
		Midway	End		
0	2	<1	<1	56 ± 6.9[a]	9.59 ± 0.37[a]
34	2	3	1	21 ± 11.6[b]	3.63 ± 0.31[b,c]
109	2	4	2	17 ± 8.7[b]	2.55 ± 0.28[b]
340	2	5	4	0	NA
18[3]	0	5	<1	67 ± 13.5[a]	3.97 ± 0.26[cr]
P-value of ANOVA				<0.001	<0.001
48-h LC50 (μg/L)[4]	12–13				

Survival and ChE activity differences for treatment groups followed by the same letter are not statistically significant ($p = 0.05$).
[1] Total amount of chlorpyrifos added to each test chamber at the beginning of the experiment.
[2] Concentration of chlorpyrifos was measured by GC.
[3] Water samples for analysis of chlorpyrifos were collected at the beginning and end of the 48-h toxicity test.
[4] 48-h LC50 for postlarvae II walleye (Phillips *et al.* 2002).

2 and 3) may seem low, these are fragile larval stages where traditional survival during culture has rarely exceeded 50% (Summerfelt 1996). In addition, comparisons of survival in the HA control with LC50 data determined by Phillips *et al.* (2002) are not valid because the program (TOXSTAT® 3.5, Western Ecosystems Technology, Cheyenne, WY, USA) used for the LC50 determination takes into consideration the survival rate of the control (no chlorpyrifos) fish.

The increased sensitivity of larval fish to toxicants may be related to development of the gills. Phillips and Summerfelt (1999) found that gill filaments were not present in walleye until 3 days posthatch, and the development of gill filaments coincides with the end of the prolarval stage when larvae make the transition from yolk sac respiration to branchial respiration. This may explain the significant increase in toxicity that occurs when walleye change from prolarvae to postlarvae I walleye. In addition, the significant increase in toxicity from postlarvae I to postlarvae II may be explained by the development of secondary lamellae that occurs at the end of the postlarvae I stage. Therefore, these findings suggest that growth and increased number of gill filaments and secondary lamellae during the first 21 days of walleye development may provide an explanation for increased sensitivity of larval fish as they shift from yolk sac respiration to branchial respiration.

Extrapolating the sensitivity of larval walleye to chlorpyrifos–HA complexes in these laboratory experiments to field conditions suggests a potential hazard from OPs adsorbed on eroded soils from fields that are treated with OPs. In addition to direct mortality, exposure from OPs may result in ChE inhibition that may cause indirect effects to organisms. For example, Beauvais *et al.* (2000) found that distance and swimming speed were both positively correlated with ChE activity for rainbow trout (*Oncorhynchus mykiss*) exposed to diazinon; thus, providing evidence for the relevance of ChE inhibition to affected organisms.

In conclusion, this study suggests that chlorpyrifos may be transferred from HA directly to fish; thus, representing an exposure route for OPs directly from suspended solids to fish. Further research is needed; however, to evaluate the bioavailability of OPs and other toxicants adsorbed on suspended solids to aquatic organisms.

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