

# Cholinergic and Behavioral Neurotoxicity of Carbaryl and Cadmium to Larval Rainbow Trout (*Oncorhynchus mykiss*)

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Received July 5, 2000; published online March 2, 2001

**Pesticides and heavy metals are common environmental contaminants that can cause neurotoxicity to aquatic organisms, impairing reproduction and survival. Neurotoxic effects of cadmium and carbaryl exposures were estimated in larval rainbow trout (RBT; *Oncorhynchus mykiss*) using changes in physiological endpoints and correlations with behavioral responses. Following exposures, RBT were videotaped to assess swimming speed. Brain tissue was used to measure cholinesterase (ChE) activity, muscarinic cholinergic receptor (MChR) number, and MChR affinity. ChE activity decreased with increasing concentrations of carbaryl but not of cadmium. MChR were not affected by exposure to either carbaryl or cadmium. Swimming speed correlated with ChE activity in carbaryl-exposed RBT, but no correlation occurred in cadmium-exposed fish. Thus, carbaryl exposure resulted in neurotoxicity reflected by changes in physiological and behavioral parameters measured, while cadmium exposure did not. Correlations between behavior and physiology provide a useful assessment of neurotoxicity.** © 2001 Academic Press

**Key Words:** cadmium; carbaryl; cholinesterase; muscarinic receptor; *Oncorhynchus mykiss*; swimming speed.

## INTRODUCTION

Many chemical contaminants in aquatic systems cause neurotoxicity as their major mode of action (e.g., most insecticides) or target the central nervous system along with other organ systems (e.g., most heavy metals). Neurotoxic injury can result in behavioral changes that may impair subsequent survival or reproduction of exposed organisms (NRC, 1992). Assessment of the neurotoxicity of a contaminant requires integration of dose-response characterization and exposure assessment. This integration relies to a large extent on data from experimental animals, including neurochemical endpoints, such as cholinesterase (ChE; includes acetylcholinesterase and other enzymes such as butyrylcholinesterase) inhibition, and behavioral endpoints

such as swimming speed. Correlation of these endpoints allows discrimination between neurotoxicity and nontoxic changes (e.g., change in a neurochemical endpoint without corresponding change in behavior) and from toxic effects not mediated through the nervous system (e.g., change in behavior without corresponding change in neurochemical endpoints). However, even with a well-known mode of action such as ChE inhibition, understanding how the physiological effect relates to behavioral alterations is limited (Costa, 1998). This limitation is especially true for fish.

Acetylcholine (ACh) is a major neurotransmitter in vertebrates, including fish (Donald, 1998). At cholinergic synapses, ChE hydrolyzes ACh, rapidly terminating signals to postsynaptic neurons as required for normal neural functioning. Contaminants that inhibit ChE may cause severe neurotoxicity, even mortality (Bhattacharya, 1993; Zinkl *et al.*, 1991). Carbaryl, a carbamate that causes acute neurotoxicity primarily through ChE inhibition, is used extensively in the United States. Agricultural use totaled  $3.5 \times 10^6$  kg active ingredient during 1988–1991; carbaryl ranked eighth among insecticides applied outdoors in urban areas in 1989–1990; and a total of 97,000 acres of forest was sprayed with carbaryl in 1992 (Larson *et al.*, 1997). Carbaryl was detected in 25% of the sites where it was monitored in United States surface waters between 1964 and 1993 (Larson *et al.*, 1997), and occurred in waters receiving agricultural and urban runoff at concentrations as high as 2.5 µg/liter (Kimbrough and Litke, 1996).

Cadmium is a more persistent neurotoxic contaminant. Globally, Cd<sup>2+</sup> has been one of the most commonly used heavy metals, with an annual production of  $1.5 \times 10^5$  metric tons during 1971–1980 (Moore and Ramamoorthy, 1984). Major uses include batteries, pigments, and electroplating. Background concentrations are approximately 0.01–0.1 µg/liter in North American rivers and lakes, while systems receiving industrial effluent have concentrations of 0.5 µg/liter or more (Moore and Ramamoorthy, 1984). At neuromuscular junctions, Cd<sup>2+</sup> interferes with Ca<sup>2+</sup>-mediated neurotransmitter release (Cooper *et al.*, 1984). In the brain, Cd<sup>2+</sup> inhibits enzymes such as Mg<sup>2+</sup>-ATPase and

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$\text{Na}^+ - \text{K}^+ - \text{ATPase}$ , causing metabolic effects and disrupting neurotransmitter uptake (Rajana *et al.*, 1990). In fish, exposure to  $\text{Cd}^{2+}$  has been found to affect brain ChE activity, causing inhibition *in vitro*, whereas *in vivo* exposure increased ChE activity (Christensen, 1975; Gill *et al.*, 1991).

A result of ChE inhibition is an increase in ACh at synapses that may cause downregulation (decrease in receptor number) of muscarinic cholinergic receptors (MChR), major cholinergic receptors in vertebrate autonomic nervous systems and brains. Downregulation of MChR in response to carbaryl and to copper has been demonstrated in rainbow trout (RBT; Jones *et al.*, 1998). Receptor downregulation is an adaptation, which, by providing fewer active receptors for ACh to stimulate, may allow return to normal behavior.

The research summarized in this paper investigated the use of three measures of cholinergic function, brain ChE activity, MChR number, and MChR affinity, in a neurotoxicity assessment of carbaryl and cadmium exposures, and correlations of those physiological measures with changes in swimming speed in larval RBT. Exposure to carbaryl or  $\text{Cd}^{2+}$  was expected to decrease ChE activity and MChR number. Correlating these physiological changes with resulting changes in swimming speed would confirm the neurotoxicity of these chemicals to RBT.

## MATERIALS AND METHODS

### Animals

RBT were obtained from the U.S. Fish and Wildlife Service Ennis National Fish Hatchery (Ennis, MT) as eyed eggs, which were hatched and cultured until swim-up in 10°C well water and then maintained at 18°C in well water until testing began. Fish were acclimated to the test temperature of 15°C for 48 h prior to exposure. Food was withheld from 2 days prior to the start of each exposure until completion of the test. Fish used in toxicity tests averaged  $32.5 \pm 1.2$  mm (total length  $\pm$  SD) for carbaryl and  $30.1 \pm 1.7$  for cadmium. Mean wet weights  $\pm$  SD were  $0.31 \pm 0.03$  g for carbaryl-exposed RBT and  $0.24 \pm 0.03$  g for RBT-exposed to cadmium.

### Toxicant Exposures

Static-renewal acute toxicity tests were conducted in basic accordance with EPA (660/3-75-009) and ASTM (E 729-88) procedures. Carbaryl was purchased from Chem Services, Inc. (West Chester, PA), and cadmium chloride was purchased from Fisher Chemicals (Fair Lawn, NJ). All other test materials and analytical reagents were purchased from Sigma (St. Louis, MO).

Carbaryl stock solutions were prepared in acetone, and exposures were conducted by addition of stock solutions to well water. Three nominal concentrations of carbaryl were

tested along with solvent (acetone) control: 188, 375, and 750  $\mu\text{g}/\text{liter}$ . The concentration of acetone in all tanks was 750  $\mu\text{l}/\text{liter}$ . Cadmium stock solutions were prepared by dissolving  $\text{CdCl}_2$  directly into ASTM Hard Water (reconstituted water: alkalinity 110–120  $\text{mg}/\text{liter}$  as  $\text{CaCO}_3$ , hardness 160–180  $\text{mg}/\text{liter}$  as  $\text{CaCO}_3$ ), and exposures were conducted in ASTM hard water. Two concentrations of  $\text{Cd}^{2+}$  were tested along with a clean ASTM water control: 2.5 and 5.0  $\mu\text{g}/\text{liter}$ . Each concentration series was replicated three times within a test. Concentrations were nominal and were chosen based on preliminary range-finding tests. Thirty RBT were randomly assigned to 15 liters of test solution in 80-liter glass aquaria. Solution renewals were done every 24 h by replacement of 7.5 liters.

### Water Quality

Water quality parameters tested included dissolved oxygen (DO), conductivity, hardness, pH, and alkalinity (Table 1; APHA, 1992). Dissolved oxygen was measured with a YSI Model 58 oxygen probe (Yellow Springs Instrument Co., Yellow Springs, OH). Alkalinity and pH were measured with a combination pH electrode Model 81-72BN and an EA 940 meter (Orion Research Inc, Cambridge, MA). Conductivity was determined with a Model 140 conductivity meter (Orion Research Inc, Cambridge, MA).

### Swimming Behavior

Ten fish in each tank were randomly sampled after 24- and 96-h exposures; at 96 h, the remaining 10 fish were transferred into aquaria containing clean water for a 48-h recovery period and then sampled. Aquaria were fitted with a polyvinylchloride circular chamber (20 cm diameter), which served as the testing arena for behavioral observations (Brewer *et al.*, 1999).

At each sample period, RBT were transferred individually into the observation chamber within each aquarium and acclimated for 5 min, and then spontaneous swimming

**TABLE 1**  
**Water Quality during Rainbow Trout Exposures to Carbaryl and Cadmium**

	Carbaryl	Cadmium
Temperature (°C)	$16.3 \pm 0.2$ (2)	$13.4 \pm 0.1$ (1)
DO (mg/liter)	$8.38 \pm 0.50$ (2)	$8.96 \pm 0.50$ (1)
PH	$8.37 \pm 0.04$ (1)	$8.09 \pm 0.07$ (1)
Alkalinity (mg/liter as $\text{CaCO}_3$ )	$258 \pm 5$ (1)	$113 \pm 3$ (1)
Hardness (mg/liter as $\text{CaCO}_3$ )	$276 \pm 4$ (1)	$163 \pm 3$ (1)
Conductivity ( $\mu\text{S}/\text{cm}$ )	$651 \pm 6$ (1)	$566 \pm 1$ (1)

*Note.* Values are means of all tanks  $\pm$  SD; there were 12 tanks for carbaryl and 9 for cadmium exposures. Number of samples/tank measured for each mean is in parentheses after each value.

behavior was videotaped for 2 min (Brewer *et al.*, 1999). These videotapes were digitized and swimming speed (cm/s) was calculated (Brewer *et al.*, 1999). After swimming was videotaped, fish were euthanized by cold shock and frozen at  $-80^{\circ}\text{C}$  until the day of the radioligand binding assay for MChR.

#### Tissue Preparation

On the day of the radioligand binding assay, brains were removed from fish of a given replicate (10 fish). The tissue was pooled ( $\sim 60$  mg) and placed in a tube with sufficient ice-cold Tris-HCl, pH 7.4, buffer (Tris 7.4) to dilute 150-fold. Tissue was homogenized for 20 s with a Tekmar Model SDT Tissuemizer (Tekmar, Cincinnati, OH) on setting 90. A 500- $\mu\text{l}$  sample was taken from this homogenate and frozen at  $-80^{\circ}\text{C}$  in a cryovial for later determination of ChE activity. Ice-cold Tris 7.4 was added to bring the final volume to 25 ml, followed by centrifugation at 47,000g for 15 min. The supernatant was discarded, and the entire process was repeated with ice-cold Tris 8.0. The resulting pellet was resuspended in 20 vol (w/v) ice-cold ultrapure  $\text{H}_2\text{O}$ , and duplicate 20- $\mu\text{l}$  samples were removed for protein determination. Tris 8.0 was added to bring the final volume of the crude membrane particulate preparation to 700 vol (w/v). Protein content of the pellet was determined by the method of Lowry *et al.*, (1951), with bovine serum albumin as a standard.

#### Radioligand Binding

The radioligand binding assay for MChR was performed as described by Jones and King (1995). Briefly, 970- $\mu\text{l}$  aliquots of tissue homogenate were incubated in tubes with increasing concentrations of *N*-[methyl- $^3\text{H}$ ]scopolamine ( $[^3\text{H}]\text{NMS}$ ; 79.5 Ci; 20  $\mu\text{l}$ ; Dupont New England Nuclear, Boston, MA) for 45 min at  $22^{\circ}\text{C}$  to measure total binding. Nonspecific binding was defined by  $[^3\text{H}]\text{NMS}$  binding in the presence of 1  $\mu\text{M}$  atropine in a parallel set of tubes. Specific binding is the difference between total binding and nonspecific binding. Incubations were terminated with rapid filtration through Whatman GF/B glass fiber filters. The filters were rinsed with 12 ml of ice-cold Tris 8.0 and transferred to vials containing 10 ml scintillation cocktail (ICN Ecolume, Irvine, CA). Radioactivity was determined by scintillation spectroscopy on a Beckman LS3801 scintillation counter (Beckman-Coulter Instruments, Fullerton, CA) at an efficiency of 46%.

#### Cholinesterase Activity

Cholinesterase activity was determined by a colorimetric method (Ellman *et al.*, 1961; Gard and Hooper, 1993; Hill and Fleming, 1982) modified for a microplate reader (Lab-

systems MultiSkan, Needham Heights, MA). Total ChE activities were measured; no attempt was made to differentiate between acetylcholinesterase and other cholinesterases. Initial experiments were done to optimize temperature and substrate concentration in RBT brain; these were  $25^{\circ}\text{C}$  and 2.5 mM acetylthiocholine, respectively. Increase in absorbance at 405 nm was monitored every 10 s for 2 min. ChE activities are reported as micromolar acetylthiocholine hydrolyzed per minute per gram tissue ( $\mu\text{M}/\text{min}/\text{g}$ ).

Homogenates were analyzed in triplicate. Analysis of a sample was repeated if the triplicate coefficient of variation (CV) was greater than 15%. With each run, a triplicate of a check standard was also analyzed. The check standard, a check on the assay conditions and reagents, was prepared earlier from pooled RBT brain homogenates and stored in cryovials at  $-80^{\circ}\text{C}$ . A run was repeated if the check standard CV was greater than 15%.

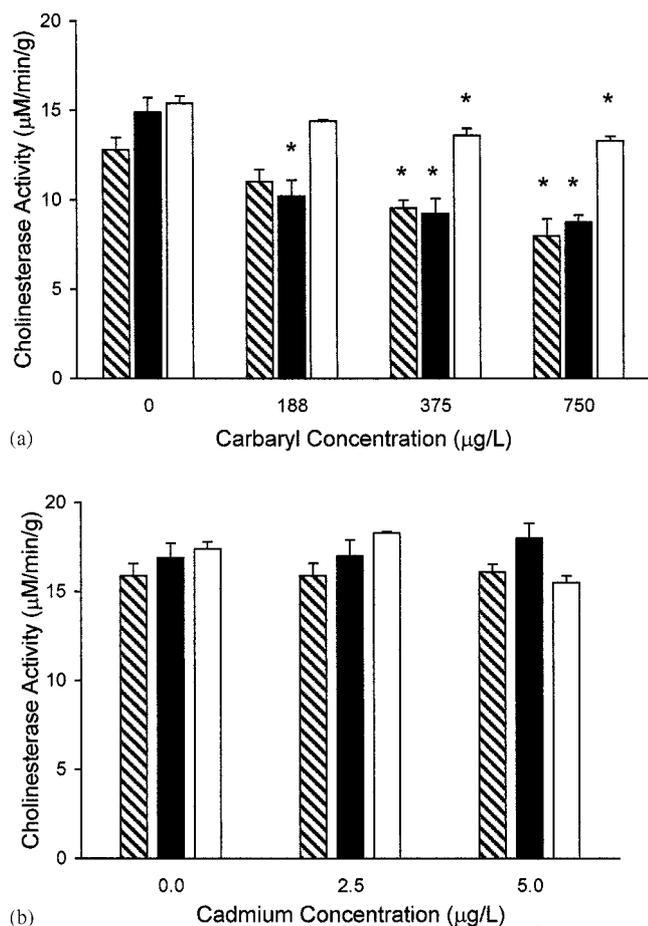
#### Data Analysis

For ChE and MChR results, analysis of variance (ANOVA) assessed differences by concentration and exposure duration, and Dunnett's multiple comparison test distinguished concentration results which differed significantly from controls by comparing each difference between means (DBM) with the minimum significant difference (MSD). Correlations between ChE activity and swimming speed were tested with linear regression. Statistical tests were done using GraphPad PRISM software (San Diego, CA). A *P* value of 0.05 was considered significant in all tests.

## RESULTS

Cholinesterase activity decreased significantly with increasing concentrations of carbaryl (two-way ANOVA;  $P < 0.0001$ ; see Fig. 1). Dunnett's comparisons between carbaryl concentrations showed that ChE activity differed significantly from control for all treatments except 188  $\mu\text{g}/\text{liter}$  following a 24-h exposure (DBM = 1.722, MSD = 1.733) and 188  $\mu\text{g}/\text{liter}$  after the recovery period (DBM = 2.519, MSD = 1.267). Carbaryl exposure duration also significantly affected ChE activity ( $P < 0.0001$ ); ChE activities were lower following both 24- and 96-h exposures than following 48-h recovery. Mean inhibition of ChE activity ranged 14–38% at 24 h, 32–41% at 96 h, and 7–14% following 48-h recovery. In contrast, ChE activity was not affected by exposure to  $\text{Cd}^{2+}$  (concentration  $P = 0.7086$ ; exposure duration  $P = 0.0955$ ).

Exposure to either carbaryl or cadmium did not significantly affect MChR binding (Fig. 2). Receptor number ( $B_{\text{max}}$ ) was unchanged with increasing carbaryl concentrations ( $P = 0.8280$ ; Fig. 2A), although there was a nonsignificant tendency for increase in  $B_{\text{max}}$  by exposure duration ( $P = 0.0669$ ), which is consistent with normal biological



**FIG. 1.** Brain cholinesterase activity in rainbow trout exposed to a toxicant for 24 h (hatched bars), 96 h (black bars), and 96 h followed by a 48-h recovery period (white bars). Each bar represents a mean  $\pm$  SE of three tanks. Asterisks indicate treatments that were significantly different from control in Dunnett's multiple comparison test. (A) Carbaryl; (B) cadmium.

variation.  $B_{max}$  had an overall mean  $\pm$  SE of  $206 \pm 6$ . Receptor affinity ( $K_D$ ) also did not vary significantly with increasing carbaryl concentrations and exposure durations ( $P = 0.5378$  and  $P = 0.9052$ ; Fig. 2B), and had an overall mean  $\pm$  SE of  $80 \pm 4$ . For RBT exposed to  $Cd^{2+}$ ,  $B_{max}$  was unchanged by concentration or exposure duration ( $P = 0.7298$  and  $P = 0.3836$ ; Fig. 2C); overall  $B_{max}$  mean  $\pm$  SE was  $131 \pm 4$ . Cadmium exposure did not affect  $K_D$  ( $P = 0.6390$  and  $P = 0.2693$ ; Fig. 2D); overall  $K_D$  mean  $\pm$  SE was  $46 \pm 2$ . Neither  $B_{max}$  nor  $K_D$  varied significantly with ChE activity for carbaryl or cadmium (data not provided).

While exposure to carbaryl caused significant changes in swimming speed, exposure to  $Cd^{2+}$  did not (Brewer *et al.*, unpublished results). Changes in swimming speed correlated positively with changes in brain ChE activity in RBT exposed to carbaryl, as revealed by linear correlations

( $r^2 = 0.7057$ ,  $P = 0.0006$ ; see Fig. 3). Swimming speed did not correlate with ChE activity in RBT exposed to  $Cd^{2+}$  ( $r^2 = 0.2256$ ,  $P = 0.1963$ ).

## DISCUSSION

Brain ChE activity in RBT was significantly inhibited by exposure to carbaryl. This result is consistent with other studies in which fish were exposed to carbaryl (Bhattacharya, 1993; Beyers *et al.*, 1994; Haines, 1981; Sambasiva Rao and Ramana Rao, 1989; Zinkl *et al.*, 1987). Of these studies, only one used RBT: adults (30–80 g) exposed in a static system for 24 h to 250 and 500  $\mu\text{g}/\text{liter}$  had mean ChE inhibition of 60.8 and 75.4%, respectively (Zinkl *et al.*, 1987). Greater ChE inhibition occurred in that study than in RBT exposed to the highest concentration of 750  $\mu\text{g}/\text{liter}$  in the current study; however, concentrations used in both studies were nominal, and it is possible that actual concentrations differed from nominal. Also, the water temperature was slightly lower ( $13^\circ\text{C}$ ) in exposures done by Zinkl *et al.*, (1987) than in the present study; the lower water temperature may have slowed detoxication of carbaryl and allowed greater ChE inhibition as a result. Furthermore, it is possible that adult RBT are more susceptible than larval RBT to ChE inhibition. The relationship between age and sensitivity to ChE inhibition by carbaryl or other anticholinesterases apparently has not been investigated in RBT. When such a relationship was investigated in adult and juvenile *Channa punctatus* exposed to the organophosphate, malathion, significant ChE inhibition occurred in juvenile fish at much lower concentrations than for adults (Dutta *et al.*, 1995). The present study demonstrated that ChE activity is an excellent indicator of neurotoxicity for carbaryl in larval RBT, as ChE activity was significantly inhibited in RBT exposed to 375 and 750  $\mu\text{g}/\text{liter}$  even after 48 h in clean water. Carbaryl concentrations in this study were higher than those detected in surface waters during routine monitoring (Larson *et al.*, 1997), though concentrations in these ranges sometimes occur in receiving waters.

Exposure to 2.5  $\mu\text{g } Cd^{2+}/\text{liter}$  or to 5.0  $\mu\text{g } Cd^{2+}/\text{liter}$  did not affect ChE activity. Few studies have correlated exposure to  $Cd^{2+}$  with effects on fish brain ChE activity. Of these, ChE activity was inhibited by *in vitro* exposures (Gill *et al.*, 1991; Huang *et al.*, 1997; Sen *et al.*, 1995), and was increased by *in vivo* exposure to  $Cd^{2+}$  (Christensen, 1975; Gill *et al.*, 1991). Following *in vivo* exposures, freshwater *Barbus conchius* exposed 48 h to 12.6  $\text{mg}/\text{liter } CdCl_2$  had a significant increase in brain ChE activity, 13% above control (Gill *et al.*, 1991), and brook trout alevins (*Salvelinus fontinalis*; 21 days posthatch) exposed for 21 days to  $Cd^{2+}$  concentrations of 0.7 or 3.43  $\mu\text{g}/\text{liter}$  had ChE activities of 46 and 60%, respectively, above control (Christensen, 1975). In contrast,  $Cd^{2+}$  inhibited ChE activities in fish brain tissues in an *in vitro* study, which found that the  $IC_{50}$  of

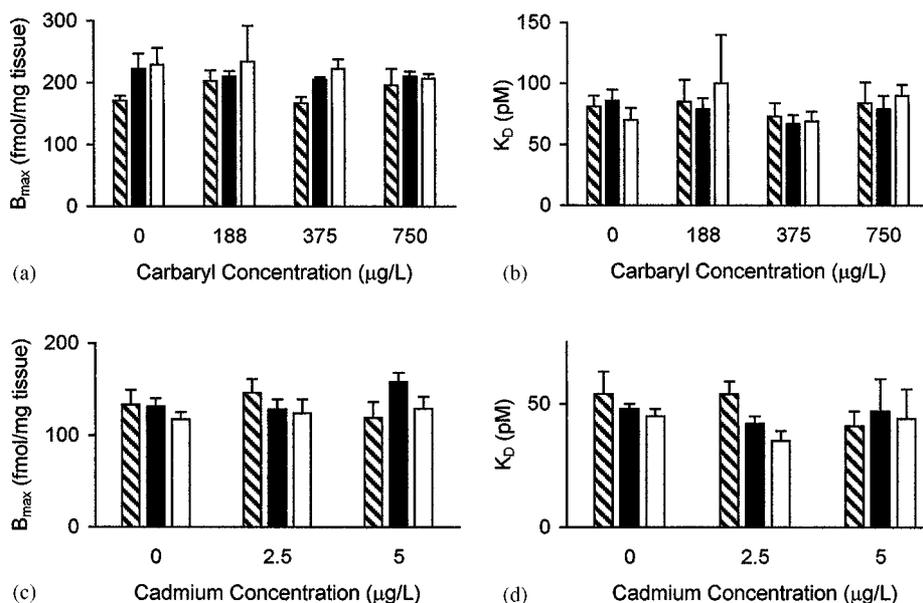


FIG. 2. Brain muscarinic receptor binding of  $N$ -[methyl- $^3\text{H}$ ]scopolamine in rainbow trout exposed to a toxicant for 24 h (hatched bars), 96 h (black bars), and 96 h followed by a 48-h recovery period (white bars). Each bar represents a mean  $\pm$  SE of three tanks. (A) Carbaryl,  $B_{max}$ ; (B) carbaryl,  $K_D$ ; (C) cadmium,  $B_{max}$ ; (D) cadmium,  $K_D$ .

$\text{CdCl}_2$  to *Channa punctatus* brain homogenates was 20  $\mu\text{M}$ , or 2.2 mg/liter (Sen *et al.*, 1995). ChE inhibition also occurred in fathead minnow muscle homogenates exposed for 20 min to  $\text{CdCl}_2$ , with an  $\text{IC}_{50}$  of 570  $\mu\text{M}$ , or 64 mg/liter (Olson and Christensen, 1980). The difference between *in vivo* and *in vitro* results may be due to the presence of metallothioneins, proteins synthesized mainly in the gills, kidney, and liver, which bind  $\text{Cd}^{2+}$  and minimize toxic insult to the organism (De Conto Cinier *et al.*, 1998; Roesijadi, 1992). Results found in the present study and in Gill *et al.* (1991) suggest that brain ChE activity in fish should not be used as an indicator of neurotoxicity resulting from exposure to  $\text{Cd}^{2+}$  until *in vivo* responses are better understood.

Organisms have several potential adaptations to ChE inhibition, including increased synthesis of ChE (Abbas and Hayton, 1997), decreased synthesis of ACh (Liu and Pope, 1996), and desensitization and downregulation of MChR (Jett *et al.*, 1993; Nostrandt *et al.*, 1997). Regression of  $B_{max}$  and  $K_D$  against ChE activity is a measure of the response of MChR to ChE inhibition. Regressions of  $B_{max}$  against ChE activity in the current study suggested that MChR did not downregulate with ChE inhibition with either of the chemicals tested. Downregulation of MChR would not necessarily be expected in RBT exposed to  $\text{Cd}^{2+}$ , in the present study, as ChE was not affected (Fig. 1), but decreases in  $B_{max}$  might be anticipated in RBT exposed to carbaryl, as seen in a previous study from this laboratory (Jones *et al.*, 1998). In that earlier study, 96-h exposure of larval RBT to

2.2 mg carbaryl/liter resulted in a significant decrease in  $B_{max}$ , whereas lower concentrations ranging 0.5–1.3 mg/liter did not cause downregulation (Jones *et al.*, 1998). However, RBT used by Jones *et al.* (1998) were larger (0.49 g) (Dwyer *et al.*, 1995) than fish exposed to carbaryl in the present study (0.31 g). A preliminary range-finding study showed that RBT of the size used would not survive concentrations above 800  $\mu\text{g/liter}$ . If exposures above 2 mg/liter had been possible with RBT used in the present study, it is possible that downregulation would have occurred. As lower concentrations were used in the present study, it is likely that ChE inhibition was insufficient to result in MChR downregulation. Jones *et al.* (1998) did not assay ChE activity, thus a relationship was not established between ChE inhibition and MChR downregulation for RBT.

The ChE inhibition observed was sufficient to affect spontaneous locomotory behaviors only in carbaryl exposures (Fig. 3). Swimming speed decreased when ChE activity decreased in RBT exposed to carbaryl. In a previous study, swimming speed also correlated with ChE activity in RBT exposed to the organophosphate insecticides, diazinon and malathion (Beauvais *et al.*, 2000). Decreased swimming speed may have serious consequences to organisms exposed in natural waters, by reducing encounters with potential food organisms, as well as impairing the ability to avoid predators, attract mates, maintain position in a school, and migrate (Little and Finger, 1990; Little *et al.*, 1993). Correlation between the readily measured physiological endpoint, ChE activity, and swimming speed provides evidence that

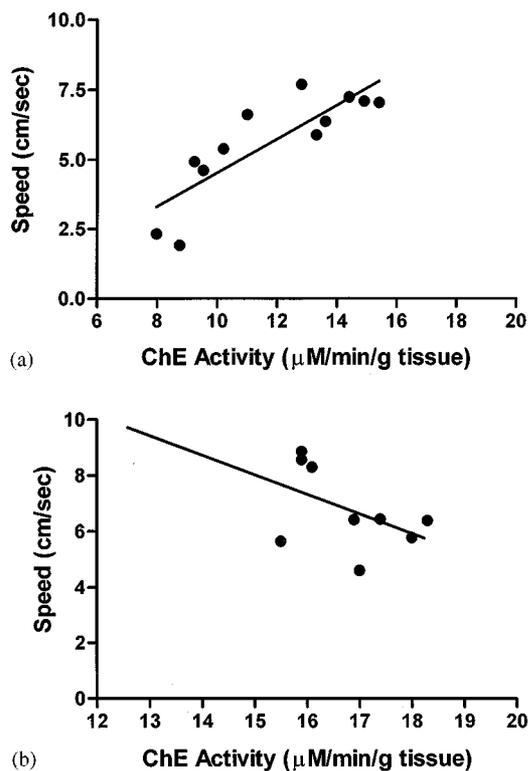


FIG. 3. Correlation of swimming speed with pooled brain cholinesterase activity in rainbow trout exposed to toxicants for 24 h, 96 h, or 96 h followed by a 48-h recovery period. Each point represents one pooled samples of 10 RBT. (A) Carbaryl ( $r^2 = 0.7057$ ;  $P = 0.0006$ ); (B) cadmium ( $r^2 = 0.2256$ ;  $P = 0.1963$ ).

when ChE inhibition is detected the organism is affected in a manner that may ultimately decrease its survival.

### CONCLUSION

Assessment of neurotoxicity of environmental contaminants is important due to extensive use and toxicity of these compounds (NRC, 1992). Inhibition of ChE is an excellent endpoint to use in the monitoring of certain neurotoxicants. This study has demonstrated that ChE inhibition correlates well with changes in swimming behavior in RBT exposed to carbaryl, though not in RBT exposed to  $Cd^{2+}$ . Biomarkers such as ChE activity are useful in identifying specific neurotoxicants and are useful only when their limitations are understood (NRC, 1992). While ChE activity has often been used as a biomarker of neurotoxicity in fish, rarely has ChE inhibition been correlated with effects at the organismal level. Information gained from the present study therefore increases the usefulness of ChE activity as a biomarker. Correlation of ChE activity with changes in swimming behavior resulting from carbaryl exposure provides evidence that when ChE inhibition is detected the organism

is affected in a manner that may ultimately decrease its survival.

### ACKNOWLEDGMENTS

This work was supported by the U.S. Army Medical Research and Material Command under Contract No. 97MM7721. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. In conducting research using animals, the investigators adhere to the laws of the United States and regulations of the Department of Agriculture. The authors thank D. Hardesty for preparation of ASTM hard water, and B. T. Johnson, J. Whyte, and three anonymous reviewers for reviewing this manuscript.

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