BEHAVIORAL AND NEUROPHYSIOLOGICAL EFFECTS OF CARBAMATE PESTICIDES ON OLFACTORY CAPABILITIES IN PACIFIC SALMON

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Introduction

The presence of pesticides within aquatic environments can induce physiological and behavioral changes in teleosts that, although sublethal, impair the survivability and ecological fitness of the organism. We are interested in carbamate pesticide effects on olfactory behaviors and physiology in the Pacific salmon, a teleost that relies heavily on olfaction for successful completion of its life history. Specific impairment of olfactory-based behaviors by pesticides has been little studied in teleosts. Among salmonids, sublethal exposure to diazinon (organophosphate) reduces the ability of Chinook (*O. tshawytscha*) to react appropriately to alarm pheromones and to home [Scholz, 2000 #109]. An important issue is the locus and mechanism by which pesticide exposure may impair olfactory behaviors. Work at the neurophysiological level in Atlantic salmon (*Salmo salar*) suggests that pesticides can act on olfactory receptor neurons (ORNs), impairing their ability to respond to odorants of biological importance (e.g. Moore 2001).

We have begun to examine more thoroughly the mechanisms involved in pesticide impairment of salmonid olfaction by establishing a baseline of carbamate effects on Coho (*O. kisutch*) at behavioral and neurophysiological levels utilizing Y-maze avoidance-behavior assays and electro-olfactrograms (EOG), a sensitive measure of multi-unit voltage potentials created by ORNs in response to odorant stimulation (e.g. Hara 1973). The carbamates chosen are compounds of current concern with moderate to high aquatic toxicity and a limited refereed database regarding their olfactory toxicity.

Methods

Experimental Animals
Juvenile Coho salmon were obtained locally at ages 3–5 months for Y-Maze experiments (mean weight= 0.97 g), and 10-15 months (mean weight= 16.0 g) for EOG experiments. Fish were maintained at natural photoperiod/temperature in flow-through tanks supplied with dechlorinated `background' water (BKD; pH 6.2–6.8, hardness 3.49 -6.19 mg/L $CaCO_3$).

Pesticide Exposures
For Y-maze experiments, fish were tank exposed for 21 days prior to behavioral testing to either carbofuran (0.075 ppm), 3-iodopropynylbutylcarbamate (IPBC, 0.048 ppm), mancozeb (1.1 ppm), or control condition in chilled (10-11°C) static 18L aquaria. Fish were fed 2% b.w. daily, and tanks were cleaned, refilled, and exposures refreshed every other day. For EOG experiments, exposures were acute (30 mins) and applied locally to the olfactory epithelium (OE). All pesticides dissolved in BKD immediately prior to use (exception: IPBC dissolved in polyethyelene glycol at 10 mg/ml; same vehicle also given to controls).

Behavioral Testing
Effects of sublethal pesticide exposure on olfactory behaviors were examined using salmonid avoidance of L-Serine (10^{-8} M; SER) in a two choice Y-trough maze (Fig. 1A; after Rehnberg et al. [1985]). Behavioral assays began with a single naïve fish in the start box with all gates closed. After 5 min. acclimation, SER was introduced into one arm of Y-maze for 5 min., then all gates were opened simultaneously. Fish were given 10 min. in maze to explore and choose an arm, after which all gates were dropped and the choice noted. The arm receiving SER was changed every second run, and the maze cleaned, in order to eliminate any bias.

Neurophysiological testing

EOGs were recorded from coho parr after Evans and Hara (1985). Individual fish were anaesthetized (2-phenoxyethanol, 0.4 ml/L), and paralyzed with intra-muscular injections of Flaxedil (2.4 mg/kg b.wt.). Fish were then wrapped in gauze, secured in a Plexiglas trough, and maintained under anesthesia by gill perfusion. After removal of skin overlying the OE, a gravity-fed stream of BKD was passed over the exposed OE into which brief pulses of odorant (10^{-5} M SER) were introduced. EOG responses to SER delivery (Fig. 2A) were recorded from the OE using an Ag/Ag-Cl electrode, then filtered, amplified, and displayed on a computer. Experimental trials consisted of the collection of pre-exposure EOG responses (PRE; n=5), followed by acute OE pesticide exposure, then post-exposure EOG responses (POST; n=5).

Results

Y-Maze Behavior

While statistical analysis has not yet been conducted on this data, it appears:

- Non-exposed control fish consistently avoid SER-scented arms
- In contrast, carbofuran- and mancozeb-exposed groups choose scented/unscented arms at a 50:50 ratio (suggesting anosmia)
- IPBC-exposed fish avoid the SER-scented arm more frequently than expected based on chance alone.

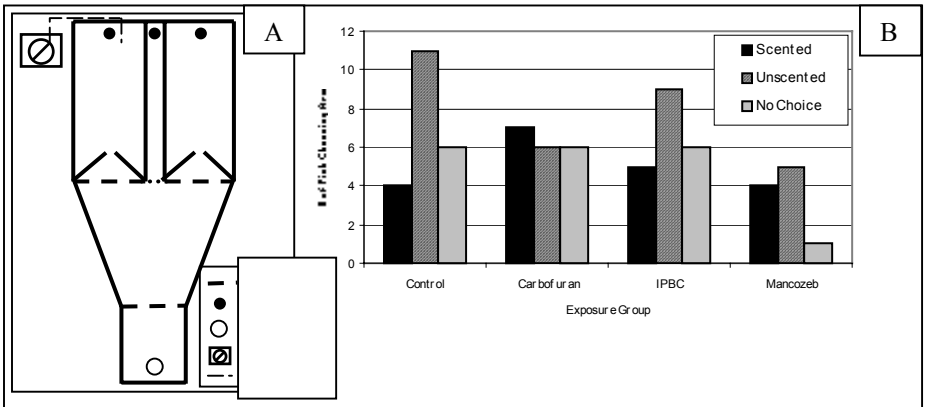
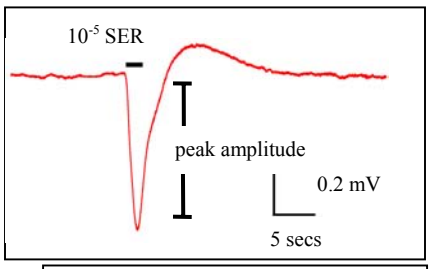


Figure 1. Y-Maze Behavioral Assay. **A.** Flow-through Y-maze apparatus. Flow: 6.4 L/min., maze length: 2.3 m. **B.** Effects of Pesticide Exposure on SER Avoidance. Plot displays number of fish choosing each arm per exposure group (carbofuran 0.075ppm, IPBC 0.048ppm, mancozeb 1.1ppm).

EOG Results

In preliminary results, significant reduction in EOG amplitude after pesticide exposure (in comparison with H2O-exposed controls, one-tailed t test, $p < 0.05$) occurred after acute 30 min. exposure:

- for carbofuran at 0.0001, 0.002, 0.01, and 2ppm
- for IPBC at 0.00048, 0.0048, and 0.048 ppm
- and, for mancozeb at 0.022, 0.22, and 2.2 ppm.



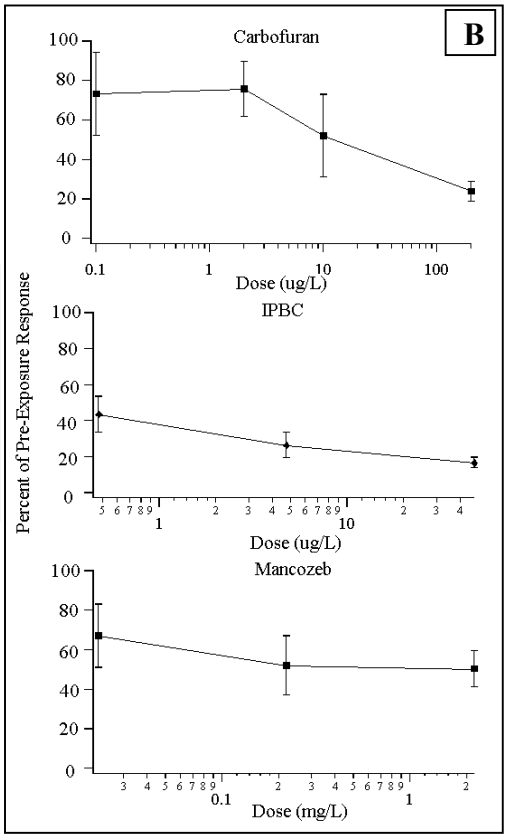


Fig. 2. Pesticide Effects on EOG **A.** EOG response to SER delivery. **B.** Dose-dependant carbamate effects on EOG amplitude. Data as POST EOG amplitude as % of PRE EOG amplitude.

Conclusions

- Carbamate pesticides interfere with salmon olfactory capabilities at both behavioral and neurophysiological levels
- This interference appears as loss of ability to avoid SER at low concentrations in Y-maze tests and as a significant decrease in the population of ORNs responding to SER in EOG responses.
- These effects appear at 50% of the 96 hr. LC_{50} for these compounds in behavioral tests (LC_{50}s: carbofuran=200ug/l, IPBC=100ug/l, mancozeb=2.2mg/L), and at several orders of magnitude below that in neurophysiological studies.
- Ongoing work is focused on establishing both a clear correlation between these different classes of effects and the mechanism by which carbamates exert their effects in the olfactory periphery.

Acknowledgements

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References

Evans, R. E. and T.J. Hara (1985). The characteristics of the electro-olfactogram (EOG): its loss and recovery following olfactory nerve section in rainbow trout (Salmo gairdneri). Brain Res **330**(1): 65-75.

Hara, T. J. (1973). Olfactory responses to amino acids in rainbow trout, Salmo gairdneri. Comp Biochem Physiol A **44**(2): 407-16.

Moore, A. and C. Waring (2001). The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (Salmo salar L.) parr. Comp Biochem Physiol B Biochem Mol Biol **129**(2-3): 269-76.

Rehnberg, BG and CB Schreck. (1987) Chemosensory detection of predators by coho salmon (Oncorhynchus kisutch): behavioral reaction and physiological stress response. Can. J. Zool. 65:481-485.

Scholz, N. (2000). Diazinon dirsupts predator. Can J of Fish Aquat Sci **57**: 1911-1918.