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Aquatic Toxicology 79 (2006) 149–157

AQUATIC TOXICOLOGY

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The alarm reaction of coho salmon parr is impaired by the carbamate fungicide IPBC

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Received 11 April 2006; received in revised form 1 June 2006; accepted 2 June 2006

Abstract

To determine whether the carbamate fungicide IPBC alters the olfactory-mediated behavioral and physiologic alarm responses of coho salmon parr (*Oncorhynchus kisutch*), groups of coho were exposed to skin extract (an alarm pheromone source) under a variety of conditions. In the 3 min following skin extract exposure, freezing behavior was significantly increased (In the 3 min following skin extract exposure, freezing behavior was significantly increased under darkness (IR lighting) but not ambient lighting (25.3 ± 2.6% and 7.5 ± 5.7%, respectively; Δ calculated as: [(time (s) after/time (s) before) − 1] × 100%), and so IR was used for further experiments. Physiologically, following skin extract exposure, plasma cortisol concentration was increased at 0.5 h (58.1 ± 14.6 ng/ml versus 4.32 ± 1.31 ng/ml, exposed versus control), hematocrit (Hct) was increased at 2 h (50.4 ± 1.0% versus 41.7 ± 1.6%), and leucocrit (Lct) was decreased at 0.5 and 2 h (0.534 ± 0.114 and 0.13 ± 0.01% versus 1.23 ± 0.20%). After 0.5 h exposures to 0, 1, 10 and 100 μg/l IPBC and skin extract, the time spent dashing (>5 cm/s) increased significantly (323 ± 118%) in the first minute after skin extract exposure, but was absent in IPBC-exposed coho. Freezing behavior increased after skin extract exposure with control and 1 μg/l IPBC exposures (11.0 ± 3.0% and 17.7 ± 11.0%, respectively), but was absent after 10 μg/l and decreased after 100 μg/l IPBC. Physiologically, Hct and plasma lactate concentration were significantly increased above controls after 1 μg/l IPBC exposure (Hct: 45.7 ± 1.6% versus 34.0 ± 1.6%, lactate: 12.8 ± 1.2 mM versus 3.30 ± 1.2 mM). After 10 μg/l exposure, IPBC alone elicited a stress response similar to skin extract. However in the 100 μg/l treatment group the stress parameters were not different from controls. These findings suggest that the behavioral and physiologic alarm responses of juvenile salmonids may be impaired by acute exposure to ≥1 μg/l IPBC.

Keywords: Coho salmon; *Oncorhynchus kisutch*; Olfaction; Alarm response; Behavior; Cortisol; Carbamate; Pesticide; IPBC

1. Introduction

For salmonids, olfaction facilitates indispensable behaviors such as migration and predator avoidance. By necessity, olfactory sensory neurons (OSNs) have a relatively unprotected environmental interface, which may leave them at risk during contaminant exposure. Environmentally realistic concentrations of metals and pesticides have been shown to impair salmonid OSNs (e.g. Hara et al., 1976; Borie et al., 1981; Waring and Moore, 1997; Baldwin et al., 2003; Jarrard et al., 2004; Sandahl et al., 2004). By impairing OSNs, contaminants may also affect olfactory-mediated behavioral and physiologic responses. For example, pesticides toxic to OSNs can lead to reduced sex steroid production (Moore and Waring, 1996a, 2001) and altered migration (Scholz et al., 2000). Compared with tests of OSN function, olfactory-mediated behavioral and physiologic response tests are more ecologically meaningful (Scott and Sloman, 2004), but they are also more technically difficult to assess.

Carbamate pesticides can rapidly impair salmonid OSNs at exposure concentrations ≤1 μg/l (Jarrard et al., 2004; Tierney et al., 2006). For example, 1 μg/l exposure to the carbamate antisapstain wood preservative IPBC (3-iodo-2-propynyl-*N*-butyl carbamate) significantly impaired coho salmon OSNs in 25 min (Tierney et al., 2006). The manner in which carbamates impair olfaction is unknown; most carbamate pesticides (i.e. *N*-methyl carbamates such as carbaryl or carbofuran) impair their targets is through addition of a carbamoyl moiety (NH_2CO) to the ser-OH esteratic site of the enzyme acetylcholinesterase. IPBC's mode of fungicidal action is unknown, although it has been hypothesized to be due to the terminal iodine (Juergensen et al., 2000). Structurally, IPBC resembles other carbamates in that it contains a core of carbamic acid (see Fig. 1), but it is not an *N*-methyl

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doi:10.1016/j.aquatox.2006.06.003

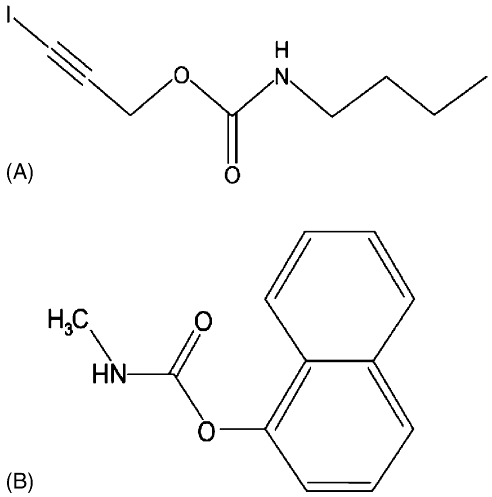

Fig. 1. The chemical structure of: (A) IPBC (3-iodo-2-propynyl-*N*-butyl carbamate) and that of the *N*-methyl carbamate and (B) carbaryl (1-naphthyl-*N*-methyl carbamate).

carbamate. IPBC has received attention in part because it can be present in runoff from lumber storage and milling sites located on salmon-producing rivers (Envirochem, 1992; Juergensen et al., 2000). Currently, the Canadian regulatory IPBC guideline for freshwater is 1.9 μg/l (CCME, 1999); US guidelines would be present on product labeling (FIFRA, 1996). The Canadian limit is well below acute lethality values for juvenile salmonids (96 h LC$_{50}$ of 67 and 95 μg/l for rainbow trout [*Oncorhynchus mykiss*] and coho salmon [*O. kisutch*], respectively [Bailey et al., 1999; Farrell et al., 1998]), but it is not below concentrations that cause olfactory toxicity (i.e. 0.1 μg/l, Jarrard et al., 2004). Although IPBC has been identified as toxic to salmonid OSNs, it is not known how this may translate to altered olfactory-mediated physiologic or behavioral responses.

This study examined the olfactory-mediated alarm responses of coho salmon parr following a sublethal IPBC exposure. The alarm response consists of physiologic and behavioral responses, characteristic of ‘fight-or-flight’ reactions (Cannon, 1914). Specifically, following sensory input, central command neurons of the autonomic nervous system facilitate physiologic stress responses (Jansen et al., 1995). These responses may include hypothalamo–sympathetic–chromaffin axis-mediated release of catecholamines (e.g. epinephrine) and hypothalamo–pituitary–interrenal axis-mediated release of glucocorticoids (e.g. cortisol) (Mommsen et al., 1999). The former providing rapid (i.e. ≥seconds) enhancement of glucose and oxygen delivery to tissues such as muscle and brain, and the latter providing a following up (i.e. ≥minutes) that also enhances glucose availability, but in addition, enhances its storage (Mommsen et al., 1999). In the teleost alarm response, both primary (e.g. cortisol increases) and secondary (e.g. glucose increase) responses typically occur (Scott et al., 2003; Rehnberg and Schreck, 1987). In non-olfactory-based stress studies, other blood sample based measures including packed red and white blood cell volume (hematocrit [Hct] and leucocrit [Lct], respectively) have been used to assess secondary physiologic stress responses. For example, Hct increased from 37 to 42% (Tierney et al., 2004a) and Lct increased from 0.57 to 0.82% (Tierney et al., 2004b) in coho salmon parr 96 h after a cold-shock (18–8 °C transfer).

Physiologic changes may underlie behavioral alarm responses, which usually involve energy expenditure. For example, when the ostariophysan matrinxã (*Brycon cephalus*) perceives alarm pheromone, a period of hyperactivity (i.e. ‘dashing’) precedes hypoactivity (i.e. ‘hiding’) (Ide et al., 2003). In salmonids, a variety of alarm response studies have reported a period of hypoactivity or increased time spent frozen in alarmed fish (in rainbow trout: Brown and Smith, 1997, 1998; Mirza and Chivers, 2001, 2003; Leduc et al., 2004; in chinook salmon (*Oncorhynchus tshawytscha*): Berejikian et al., 1999, 2003; Scholz et al., 2000). As the magnitude of the alarm signal increases, the amount of inactivity increases (Mirza and Chivers, 2003). Thus far, dashing behavior has not been observed in coho salmon.

In the present study, swimming behaviors were observed and primary and secondary measures of stress were taken in coho salmon parr following skin extract exposure. Typically, salmonid alarm response behavior is examined in individuals; however, we chose to measure it in groups, which may improve ecological relevance. This also facilitates physiologic measures, which tend to be measured in five or more tank-exposed fish. To help limit any non-olfactory-based alarm reaction (e.g. visual alarm reaction propagation; Smith, 1992), a novel infrared camera and lighting system was designed to allow testing under darkness. To help resolve a suitable sampling time and skin extract concentration, the physiologic responses were measured at 0, 0.5, 1 and 2 h after exposure to three concentrations of skin extract. Finally, both the physiologic and behavioral responses of coho exposed to skin extract were characterized following exposure to three concentrations of IPBC.

2. Materials and methods

2.1. Animals

Coho salmon parr were obtained from the Fisheries and Oceans Canada (DFO) Capilano Fish Hatchery (North Vancouver, BC) in January 2005 ($n = 370$, mass 10.0 ± 0.13 g, length 10.0 ± 0.04 cm, fork length condition factor 0.995 ± 0.006; Adams et al., 1993). Coho were held for at least 3 weeks prior to experimentation in a 500 l flow-through tank supplied with dechlorinated municipal water (dissolved oxygen > 90% saturation, pH 6.8, hardness 6.12 mg/l CaCO$_3$), using a 12-h light:12-h dark photoperiod, and were fed commercial salmon feed *ad libitum* (EWOS Pacific, Surrey, BC).

2.2. Skin extract and pesticide preparation

Skin extract was prepared as in Brown and Smith (1998). In brief, fish were sacrificed by cervical dislocation, the skin was then removed, rinsed with distilled deionized water (DDW), massed and placed on ice. In the first experiment with lighting, 50 mg/l of skin extract was used as this concentration was comparable to that used in a similar study with rainbow trout (i.e.