Effects of dietary exposure to the pyrethroid pesticide esfenvalerate on medaka (Oryzias latipes)

Ingeborg Werner\textsuperscript{a,}\textsuperscript{*,} Juergen Geist\textsuperscript{a,b}, Mark Okihiro\textsuperscript{a}, Philipp Rosenkranz\textsuperscript{a}, David E. Hinton\textsuperscript{c}

\textsuperscript{a}School of Veterinary Medicine, Department of Anatomy, Physiology and Cell Biology, University of California, Davis, CA 95616, USA
\textsuperscript{b}Fachgebiet für Wildbiologie und Wildtiermanagement, Technische Universität München, Germany
\textsuperscript{c}Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

Abstract

The pyrethroid insecticide esfenvalerate is widely used on orchard crops throughout California. In the aquatic environment, this compound is likely to accumulate in sediments, food particles and benthic organisms due to its lipophilicity and environmental persistence. This pilot project tested the hypothesis that esfenvalerate is toxic to medaka (Oryzias latipes) when taken up with the diet. For 7 days fish were fed diets, which contained esfenvalerate in three different concentrations (4, 21, 148 mg/kg, measured). Endpoints measured were mortality, fecundity, fertilization and hatching success of embryos, viability of larvae and cellular stress protein (hsp60, hsp70, hsp90) levels. The toxicity of aqueous exposure of medaka to esfenvalerate was also determined. Whereas the 96-h \textit{LC\textsubscript{50}} in the aqueous exposure was $< 9.4 \, \text{mg/l}$, the dietary exposure did not cause mortality. Possible effects of dietary esfenvalerate were seen on fertilization and hatching success and the number of non-viable larvae. Expression of hsp60 and hsp90 showed a dose-dependent response pattern. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Pyrethroid pesticides; Esfenvalerate; Medaka; Dietary exposure; Reproductive toxicity; Stress proteins
1. Introduction

With increasing concerns regarding environmental health and safety of organophosphate insecticides (OPs), and increasing resistance of pest organisms to OPs, the use of pyrethroid insecticides, such as esfenvalerate (Asana®), is rising at a fast rate (Epstein, Bassein, & Zalom, 2001). Esfenvalerate is hydrophobic ($K_{ow} = 6.2$), relatively persistent (half-life = 66–287 days) and highly toxic to many aquatic species (US EPA, 2001). While bivalves are relatively insensitive to pyrethroids, and can bioaccumulate these compounds [bioconcentration factor (BCF) of fenvalerate in oysters: $>4700$; Clark et al., 1989], pyrethroid toxicity to fish is approximately one order of magnitude greater than that of OP compounds (Extonet, 2001; Haya, 1989). Toxicity, and therefore bioavailability, of sediment sorbed pyrethroids to aquatic invertebrates has been demonstrated (Clark et al., 1989). Bioaccumulation of such compounds by benthic clams, and subsequent dietary uptake may therefore be one of the most important routes of pyrethroid exposure for bivalves and bottom feeding fish species (Williams, Brooke, Matthiesen, Mills, Turnbull, & Harrison, 1995).

Although concentrations of hydrophobic organic compounds can be orders of magnitude higher in food organisms than in the water column, very little information is available on the toxic effects of dietary pyrethroids on fish. Our pilot study determined the sensitivity of medaka (O. latipes) to esfenvalerate in solution, and investigated the effects of dietary exposure to this compound. We chose various reproductive endpoints (fecundity, fertilization and hatching success, larval viability) and expression of three groups of stress proteins (hsp60, hsp70, hsp90) to measure exposure and effect. Stress proteins are considered to be general indicators of sub-lethal cellular protein damage. In addition, the three hsp groups, predominantly hsp90, are associated with steroid hormone receptor activation (Feige, Morimoto, Yahara, & Polla, 1996).

2. Materials and methods

2.1. Medaka aqueous exposure

Medaka (4.5 months old) were exposed to 1.5 l of 0.1, 1 and 10 $\mu$g/l (nominal) esfenvalerate and 0.05% methanol (solvent control) using three replicate 2-l beakers containing 10 (5 male, 5 female) fish per treatment. The test was performed at 22.7–22.9 °C, dissolved oxygen (DO): 85.1–92.4%, and 16 L:8 D. Fish were not fed. Water (75%) was renewed daily. After 96 h, surviving fish were counted, and samples preserved for chemical analysis.

2.2. Medaka dietary exposure

Experimental diets (MeOH control, 3, 30, 300 mg/kg (nominal) esfenvalerate) were prepared by mixing MeOH, and esfenvalerate into standard PC-diet used at our
medaka colony. Fish (6 males, 6 females per tank) were fed experimental diets in 20-l flow-through aquaria. Water temperature was 25 °C (±1 °C), pH: 7–8, conductivity: 280–320, ammonia/nitrite: 0, nitrates: <10 mg/l, and DO: 6–8 mg/l. Fish were fed twice daily at 5% of body weight. Mortality and egg production was recorded twice daily. After 7 days, medaka were frozen in liquid N₂ and stored at −80 °C.

2.3. Egg collection and processing

On day 6, eggs produced during the previous 24 h were collected, manually separated, then disinfected in 25 g/l NaCl for 10 min. Eggs were counted and maintained in control water at 25 °C. Water was changed daily and each batch assessed for: no. dead eggs, no. unfertilized eggs, no. hatched eggs, and no. live and dead larvae.

2.4. Stress protein analysis

Hsp proteins were analyzed by Western blotting. Whole animals were homogenized in tris buffer (pH 7.2). Subsamples of equal total protein content (50 µg) were separated by SDS–PAGE, then electroblotted onto Immobilon-P membrane. Monoclonal hsp70 and hsp90 antibodies (Affinity Bioreagents MA3–001, StressGen SPA-830), and a polyclonal hsp60 antibody (StressGen SPA-805) were used as a probes. Bound antibody was visualized by chemiluminescent reagents (Tropix Inc.), and hsp bands quantified by densitometry.

2.5. Chemical analysis

Water samples (300 ml) were filtered through an activated charcoal column, eluted with 1 ml MeOH and analyzed for esfenvalerate by ELISA (Shan, Stoutamire, Wengatz, Gee, & Hammock, 1999) and GC–MS. Diet samples were extracted (Schimmel, Garnas, Patrick, & Moore, 1983), then analyzed by ELISA.

2.6. Statistical analysis

Results were analysed by one-way ANOVA and t-test.

3. Results and Discussion

Measured esfenvalerate concentrations in aqueous exposure solutions were 0.19, 0.86 and 9.4 µg/l; concentrations in experimental diets were 4, 21 and 148 mg/kg. Experimental concentrations were chosen based on known 96 h-LC₅₀ data for other fish species for aqueous exposure, and on tissue concentrations measured in esfenvalerate exposed clams for dietary exposure (unpublished data, this laboratory). One higher and one lower concentration were included in each set of experiments. Mortality of medaka reached 100% after 4 days of aqueous exposure to 9.4 µg/l esfenvalerate. All fish survived the 4-day exposure to 0.86, 0.19 µg/l esfenvalerate and
control water. No mortality was observed during the dietary exposure to as much as 148 mg/kg esfenvalerate.

Table 1 shows results on medaka fecundity, fertilization and hatching success of embryos produced on day 6 of the dietary exposure. Generally, statistical power of this pilot project was low due to the small sample size ($n = 3$). In addition, the exposure period of 7 days was relatively short. There was a downward trend in fecundity and fertilization success with increasing esfenvalerate concentration, but it is likely that methanol contributed, at least in part, to the observed effects. An increasing trend ($P < 0.1$) was observed with increasing insecticide concentrations in the percentage of non-viable and dead larvae.

Stress proteins hsp60 and hsp90 appear to be good indicators of esfenvalerate exposure (Fig. 1). Hsp60 was significantly ($P \leq 0.05, P \leq 0.1$) elevated in females of the 21 and 148 mg/kg groups. Hsp 90 was significantly elevated in males of the 148 mg/kg group and showed an increasing trend ($P \leq 0.1$) in females of the 21 and 148 mg/kg groups.

Based on our data, it can be assumed that dietary exposure to esfenvalerate may cause deleterious reproductive effects, in particular on hatching success and larval viability. Schulz and Liess (2001) measured sublethal effects of low fenvalerate doses (1–10 ng/l, 1 h) on caddisfly larvae, and detected significant reductions in emergence success and adult weight. Although dietary esfenvalerate doses given in this experiment were much higher, the little information we have on BCFs in bivalves renders such concentrations plausible. Samsoe-Petersen et al. (2001) measured esfenvalerate concentrations of 9 µg/kg dry weight in sediments of a pond sprayed with the pesticide at a rate of application of 25 g/ha. If BCFs in bivalves are on the order of $10^3$ as reported for oysters (>4700) (Clark et al., 1989), exposure concentrations of this experiment may well be within an environmentally realistic range. In addition, the relatively long half-life of esfenvalerate can lead to exposure periods that are much longer than our 7-day experiment. However, data on pyrethroid concentrations in the aquatic environment is scarce. Clearly, more monitoring and laboratory studies are needed to adequately assess the environmental impacts of pyrethroid pesticides.

### Table 1
Fertilization and hatching success of eggs produced by medaka exposed to dietary esfenvalerate for 7 days. Data are shown as mean ± standard error ($n = 3$). A, B, C indicate statistically significant differences ($P \leq 0.05$); (A,B,C) indicate trends ($P \leq 0.1$).

<table>
<thead>
<tr>
<th>Diet treatment</th>
<th>Total number of eggs</th>
<th>Fertilized eggs (%)</th>
<th>Dead eggs (%)</th>
<th>Hatched eggs (% of total)</th>
<th>Hatched eggs (% of fertilized)</th>
<th>Non-viable larvae (% of total)</th>
<th>Non-viable larvae (% of fertilized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH–control</td>
<td>67.3±24.2 AB</td>
<td>98±2.5</td>
<td>4.0±2.3</td>
<td>83.7±8.7 A</td>
<td>85.8±6.4</td>
<td>6.4±2.3 A</td>
<td>6.6±2.3 A</td>
</tr>
<tr>
<td>Esfen 4 mg/kg</td>
<td>103±9.8 A</td>
<td>97±1.9</td>
<td>7.8±1.7</td>
<td>85.1±2.3 AB</td>
<td>88±1.2</td>
<td>3.2±0.2 A</td>
<td>3.3±0.2 A</td>
</tr>
<tr>
<td>Esfen 21 mg/kg</td>
<td>71.3±3.9 AB</td>
<td>82±10.6</td>
<td>3.7±1.2</td>
<td>61.2±10.4 ABC</td>
<td>74.9±8.7</td>
<td>16.4±10.4 ABC</td>
<td>20±10.4 ABC</td>
</tr>
<tr>
<td>Esfen 148 mg/kg</td>
<td>42.3±18.2 B</td>
<td>87±7.5</td>
<td>0.8±5.7</td>
<td>62.2±10.1 AC</td>
<td>71.8±15.5</td>
<td>20.5±13.3 BC</td>
<td>23.6±12.7 BC</td>
</tr>
</tbody>
</table>
Fig. 1. Stress protein (hsp60, hsp70, hsp90) levels in male and female medaka after 7-day dietary exposure to esfenvalerate. Results of western blots are shown as average relative density of hsp bands ± standard error (n = 3). * = P ≤ 0.05; (*) = P ≤ 0.1.

Acknowledgements

We much appreciate the assistance of Michelle Hornberger and Dan Cain, USGS Menlo Park, CA, in collecting clams, and of Guomin Shan, UC Davis, in chemical analyses. Funding was provided, in part, by EPA contract R826940–01–1.
References


