Acute Toxicity to Freshwater Organisms of Antiparasitic Drugs for Veterinary Use

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ABSTRACT: The acute toxicity of five antiparasitic drugs used in the veterinary field—amprolium hydrochloride (APH), bithionol (BT), levamisole hydrochloride (LVH), pyrimethamine (PYM) and trichlorfon (TRC)—to the aquatic organisms *Oryzias latipes*, *Daphnia magna*, and *Brachionus calyciflorus* was examined. The toxicity test with *O. latipes* was conducted in accordance with the OECD Guidelines for the Testing of Chemicals (1993) to determine the 24-, 48-, 72-, and 96-h LC$_{50}$ values. In addition, 24- and 48-h EC$_{50}$ values for *D. magna* and a 24-h EC$_{50}$ for *B. calyciflorus* were determined with the DAPHTOXKIT F™ magna (Creasel, Belgium) and the ROTOXKIT F™ (Creasel, Belgium), respectively. High-performance liquid chromatographic analysis revealed that APH, LVH, and PYM were stable in water, but BT was unstable, decreasing by 84% on average at 24 h. TRC rapidly decomposed, with only 0.7% of the initial concentration remaining after 96 h, forming dichlorvos. The toxicity of TRC to *O. latipes* was determined in two ways: exposure to the same medicated water for 96 h (static test) and exposure to medicated water replaced every 24 h (semistatic test). AMP, LVM, and PYM were tested in the static condition, and BT was tested in the semistatic condition. BT was most toxic to *O. latipes*, with a 96-h LC$_{50}$ of 0.24 mg L$^{-1}$, followed by PYM, with a 96-h LC$_{50}$ of 5.6 mg L$^{-1}$. The 24-, 48-, 72-, and 96-h LC$_{50}$ values of TRC in the static test were 92.0, 45.2, 29.5, and 17.6 mg L$^{-1}$, respectively, which tended to be lower than those in the semistatic test, especially late in the observation period. *D. magna* was the most susceptible to TRC, with a 48-h EC$_{50}$ as low as 0.00026 mg L$^{-1}$. The 48-h EC$_{50}$ values of BT, PYM, and LVH for *D. magna* were 0.3, 5.2, and 64.0 mg L$^{-1}$, respectively. *B. calyciflorus* was the most susceptible to BT, with an EC$_{50}$ of 0.063 mg L$^{-1}$, followed by PYM, with an EC$_{50}$ of 15.0 mg L$^{-1}$. Among the test compounds, APH was the least toxic to all the freshwater organisms tested, with a 96-h LC$_{50}$ of $>600$ mg L$^{-1}$ for *O. latipes*, a 48-h EC$_{50}$ of 227 mg L$^{-1}$ for *D. magna*, and an EC$_{50}$ of 403 mg L$^{-1}$ for *B. calyciflorus*. © 2005 Wiley Periodicals, Inc. Environ Toxicol 20: 60–66, 2005.

Keywords: acute toxicity; *Oryzias latipes*; *Daphnia magna*; *Brachionus calyciflorus*; amprolium hydrochloride; bithionol; levamisole hydrochloride; pyrimethamine; trichlorfon

INTRODUCTION

A variety of chemicals, including antimicrobial agents, anthelmintics, insecticides, vitamins, digestive medicines, and vaccines, have been utilized as pharmaceuticals in animal husbandry. Most frequently used are antimicrobial agents, followed by antiparasitic drugs consisting of anthelmintics and insecticides. In Japan, antimicrobial agents and antiparasitic drugs accounted for 38.1% (29,248 million yen) of all the veterinary medicinal products sold in 2002 (Food Safety and Consumer Bureau, 2003). Unlike human pharmaceutical products, antimicrobial agents and antipar-
asitic drugs for veterinary use are administered to all animals of the same herd or flock for purposes not only of curing infected animals but also of preventing infections and promoting growth; thus, the amount consumed is an environmental concern. After administration to animals, these drugs are excreted intact or with their metabolites, accumulating in waste. Animal dung is spread on cultivated fields as compost, releasing these chemicals into the environment (Ternes, 1998; Hirschl et al., 1999), and liquid excreta enter aquatic systems through sewage treatment plants (Harada, 1997). In Japan compost made of animal manure is also spread on paddy fields, habitats of aquatic organisms such as fish, cladocerans, amphibians, and insects. Okamura et al. (1999) and Manosa et al. (2001) described the ecotoxicological risk to aquatic organisms of using pesticides in paddy fields.

Toxicity testing in aquatic organisms has been carried out mainly with algae, cladocerans, fish, protozoa, and rotifers, as bioindicators (Girling et al., 2000), and several relevant articles on antimicrobial agents have been published (Dojmi di Delupis et al., 1992; Brambilla et al., 1994; Migliore et al., 1997; Holten Lützhoft et al., 1999; Halling-Sørensen, 2000; Wollenberger et al., 2000). However, except for ivermectin, which was found to reduce the population of dung-degrading insects in pastures, threatening the human environment (Wall and Strong, 1987; Written, et al., 1993; Lumaret and Errouissi, 2002), there has been little data thus far on the toxicity of antiparasitic drugs, although concern has been expressed about their environmental toxicity (McKellar, 1997; Williams, 1997). Given the acute LD₅₀ values found in laboratory animals (Holmstedt et al., 1978; Yoshimura et al., 1985; Milne, 2002), antiparasitic drugs are thought to be more toxic to some classes of environmental organisms than are antimicrobial agents.

We report here the results of the present study, in which we tested the acute toxicity of antiparasitic drugs to the killifish (*Oryzias latipes*), the cladoceran (*Daphnia magna*), and a rotifer (*Brachionus calyciflorus*) to determine their possible degrees of toxicity. We also discuss (1) that our findings on the toxicity of antiparasitic drugs to aquatic organisms could be correlated with that to mammals reported in the literature and (2) that some antiparasitic drugs might have adverse effects on aquatic organisms.

**MATERIALS AND METHODS**

**Test Compounds**

Five antiparasitic drugs were used: amprolium hydrochloride (APH), bithionol (BT), levamisole hydrochloride (LVH), pyrimethamine (PYM), and trichlorfron (TRC). APM, BT, LVH, and PYM were purchased from Sigma Chemical Company (St. Louis, MO, USA). TRC used was Negufon® (Japan-Bayer Agro-Chem, Tokyo, Japan), which is 97% pure. The purity of the other four antiparasitic drugs was greater than 99%. Potassium dichromate (purity, 99.5%) was used as a reference substance and dichlorvos (DDVP; purity, 98%) as a standard substance in order to estimate the changes to DDVP; they were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). APH is widely used for the treatment and prevention of coccidiosis in chickens. BT is an anthelmintic exclusively active against *Fasciola* species in ruminant animals. LVH is an anthelmintic effective against nematode infections in cattle, pigs, and chickens. PYM is used for the control and prevention of toxoplasmosis in pigs and also is added to the feed of chickens in order to prevent avian leucocytozoonosis. TRC, an ecto- and endoparasiticide, is used in such animals as cattle, pigs, and chickens and also is sprayed in and around the animals’ shelters.

**Test Waters**

Test compounds were dissolved in dechlorinated tap water for the toxicity test with *O. latipes*, or in freshwater attached to the test kits for the toxicity tests with *D. magna* and *B. calyciflorus*. APM, LVH, and TRC were directly dissolved in these waters. BT hardly dissolves in water (0.4 mg L⁻¹; Merck Index, 11th ed.), so it was dissolved mechanically in the test water in a conical flask with a magnet stirrer (approximately 3 h) and an ultrasonic apparatus, filtered through a Millicupe®-HV (0.45 μm; Millipore Corporation, Bedford, MA, USA), and analyzed by high-performance liquid chromatography (HPLC) before being diluted to the desired concentrations for exposure to the test organisms. The dissolving of BT in the conical flask and HPLC analysis were performed in the dark. PYM was first dissolved in ethanol and then diluted in water. The proportion of ethanol to water did not exceed 0.002. The total hardness of dechlorinated tap water was 130 ± 10 mg CaCO₃ L⁻¹ (Drop Test; Kyoritsu Chemical-Check Lab. Corp., Tokyo, Japan). The initial pH of the dechlorinated water was 7.4 ± 0.2. The initial pH of the freshwater was 7.9 ± 0.1 and 8.1 ± 0.1 in the tests with *D. magna* and *B. calyciflorus*, respectively, but change in pH was not monitored during the test. The pH of the water was measured with a model F-23 (Horiba Ltd., Kyoto, Japan).

**Stability in Water**

The stability of the test compounds in dechlorinated tap water was examined by HPLC. Test compounds were dissolved in 7 L of dechlorinated water stocked with some *O. latipes* in a glass aquarium (29 cm in diameter, 13.5 cm in depth). The water temperature was 23°C ± 1°C. Fluorescent lamps provided light intensity ranging from 200 to 300 lux at the water surface, with a 12 h:12 h (light:dark) photoperiod. The medaka dead were removed. The HPLC
TABLE I. Wavelength, mobile phase, and retention time under analysis conditions of each compound

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength (nm)</th>
<th>Mobile Phase</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amprolium hydrochloride</td>
<td>264</td>
<td>0.2 mol L(^{-1}) KH(_2)PO(_4)–acetonitrile (90:10), added 1-hexanesulfonic acid sodium salt in a concentration of 0.05 mol L(^{-1})</td>
<td>12.5</td>
</tr>
<tr>
<td>Bithionol</td>
<td>210</td>
<td>H(_2)O–acetonitrile–phosphoric acid (30:70:0.1)</td>
<td>8.9</td>
</tr>
<tr>
<td>Levamisole hydrochloride</td>
<td>214</td>
<td>0.01 mol L(^{-1}) KH(_2)PO(_4)–acetonitrile (85:15)</td>
<td>6.1</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>209</td>
<td>0.01 mol L(^{-1}) KH(_2)PO(_4)–acetonitrile (80:20)</td>
<td>18.2</td>
</tr>
<tr>
<td>Trichlorfon</td>
<td>200</td>
<td>0.01 mol L(^{-1}) KH(_2)PO(_4)–acetonitrile (75:25)</td>
<td>5.7 for trichlorfon</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.3 for DDVP</td>
</tr>
</tbody>
</table>

An approximately 10-mL water sample was collected at appropriate intervals. The concentrations of APH, LVH, and PYM at the start of the stability test were 100, 17.7, and 4.91 mg L\(^{-1}\), respectively, and the analyses were performed for water samples collected at 24, 48, 72, and 96 h. BT analysis initially was performed in three concentrations, 0.126, 0.242, and 0.483 mg L\(^{-1}\), and changes in concentration were analyzed for water samples collected at 2, 4, 6, and 24 h. TRC was dissolved in a concentration of 25 mg L\(^{-1}\), and the concentrations of TRC and DDVP were determined for water samples collected at 0, 2, 4, 6, 24, 48, 72, and 96 h. Analyses were performed immediately after collection of samples.

Toxicity Testing with Orgziyas Latipes

Fish acute toxicity tests were conducted in accordance with the OECD Guideline for Testing of Chemicals (OECD, 1992) using adult O. latipes (length 28 ± 2 mm) purchased from a local fish farm. A group of 10 fish was exposed to different concentrations of test compounds dissolved in 7 L of dechlorinated tap water in a glass aquarium (29.5 cm in diameter, 13 cm in depth) for 96 h under fluorescent illumination of 200–300 lux at the water surface, with a 12 h:12 h (light:dark) photoperiod. The dissolved oxygen concentration was above 80% of air saturation in any test. A model OM-12 dissolved oxygen meter (Horiba Ltd., Kyoto, Japan) was used. Water temperature was 23°C ± 1°C. Except for test compounds shown to be decomposed in water, test solutions were unchanged and exposed to O. latipes for 96 h (static test). For test compounds found to be unstable in water, test solutions were renewed every 24 h (semistatic test). Mortality was recorded at 24, 48, 72, and 96 h. All tests were performed in triplicate, and the concentrations that produced 50% mortality of the test organisms (LC\(_{50}\)) and their 95% confidence limits were calculated by probit analysis on the basis of nominal concentrations, using the Eco-Tox R1.1, recommended by the Japanese Society of Environmental Toxicology.

Toxicity Testing with Cladoceran

Twenty-four- and 48-h toxicity tests with D. magna were conducted using the DAPHTOXKIT F\(_{12}\) magna (Creasel, Belgium), which performs an acute toxicity test with D. magna Straus in accordance with the test procedures prescribed by the OECD (1984) and ISO (1996) guidelines. Tests were performed according to the manufacturer’s instructions. Test plates inoculated with D. magna were incubated at 20°C ± 1°C in the dark, and immobilization of D. magna after exposure to the toxicants was used as a toxicity end point. The neonates inoculated into the test plates were not older than 8 h after hatching. All tests were performed at least twice in the static condition, and the 24- and 48-h concentrations that produced 50% immobilization of the organisms (EC\(_{50}\)) and their 95% confidence limits were calculated by probit analysis on the basis of nominal concentrations, using the Eco-Tox R1.1. The 24- and 48-h EC\(_{50}\) values of K\(_2\)Cr\(_2\)O\(_7\), used as a reference, were 1.08 and 0.86 mg L\(^{-1}\), respectively, with 95% confidence limits of 0.94–1.36 and 0.71–0.93 mg L\(^{-1}\), respectively (n = 2).

Toxicity Testing with Rotifer

Twenty-four-hour toxicity tests with B. calyciflorus were conducted using the ROTOXKIT F\(_{12}\) (Creasel, Belgium). The test was carried out according to the manufacturer’s instructions. Test plates inoculated with B. calyciflorus were incubated at 25°C in the dark, and immobilization of B. calyciflorus after exposure to the toxicants was used as a toxicity endpoint. The rotifers transferred into the test wells were within 2 h of cyst hatching. All tests were performed in triplicate, and the 24-h EC\(_{50}\) values and 95% confidence limits were calculated by probit analysis on the basis of nominal concentrations, using the Eco-Tox R1.1. The EC\(_{50}\)
of K₂Cr₂O₇, used as a reference, was 12.1 mg L⁻¹, with 95% confidence limits of 10.5–14.0 mg L⁻¹ (n = 2).

RESULTS

Stability in Water

As shown in Figure 1, APH, LVH, and PYM were shown to be very stable and not decomposed in dechlorinated water, even after 96 h. However, BT was unstable for light, decreasing by 84% on average at 24 h (Fig. 2). TRC was rapidly decomposed, with the concentration at 96 h as low as 0.175 mg L⁻¹ (0.7% of the initial concentration), replacing DDVP (Fig. 3).

Toxicity to Aquatic Organisms

Table II shows the 24-, 48-, 72-, and 96-h LC₅₀ values of five antiparasitic drugs for O. latipes. No LC₅₀ of APH could be determined because of its nonlethality, even at 600 mg L⁻¹. As BT was considered unstable in water, the toxicity testing of O. latipes was carried out under semistatic conditions. TRC toxicity was determined under both static and semistatic conditions, which showed a tendency of the LC₅₀ to be lower in the static test than in the semistatic test, especially late in the observation period. Spasmodic twitching of the body and immobilization occurred in O. latipes exposed to toxic levels of TRC, probably because of the cholinesterase-inhibiting activity of organophosphate compounds. Among the test compounds, TRC had the highest LC₅₀ at 24 h, greater than LVH, PYM, and BT. The longer the exposure, the lower was the observed LC₅₀ of TRC, but not of BT, LVH, and PYM. PYM produced hyperactive behavior and loss of equilibrium in O. latipes before death. The magnitude of the change in pH during any test was always less than 1 pH unit.

APH was less toxic to D. magna, which had a 48-h EC₅₀ of 227 mg L⁻¹, and B. calyciflorus, whose EC₅₀ was 403 mg L⁻¹, followed by LVH, with a 48-h EC₅₀ of 64.0 mg L⁻¹ and an EC₅₀ of 98.9 mg L⁻¹, respectively (Tables III and IV). Although not observed for O. latipes, the EC₅₀ of PYM for D. magna at 48 h was about one-half that at 24 h. TRC was the most toxic to D. magna, with a 48-h EC₅₀ as low as 0.00026 mg L⁻¹, followed by BT, with a 48-h EC₅₀ of 0.30 mg L⁻¹. BT was the most toxic to B. calyciflorus, for which the EC₅₀ was about fivefold lower than the 48-h EC₅₀ for D. magna. PYM was more toxic than LVH to both D. magna and B. calyciflorus, as observed for O. latipes.

DISCUSSION

According to Canton and Van Esch (1976), the EC₅₀ values of APH and PYM for D. magna were 230 and 4.8 mg L⁻¹,
respectively. These values are in fairly good agreement with those in the present study. However, there seemed to be differences in susceptibility to APH among fish species, as the LC$_{50}$ for Poecilia reticulate was 270 mg L$^{-1}$, whereas it was 1550 mg L$^{-1}$ for Oncorhynchus mykiss (Canton and van Esch, 1976) and in the present study it was >600 mg L$^{-1}$ for O. latipes. It is likely that O. latipes is more sensitive to LVH than is Anguilla anguilla, as the 24-h LC$_{50}$ of LVH was 45.3 mg L$^{-1}$ for O. latipes, in the present study, but 250 mg L$^{-1}$ for A. anguilla (Taraschewski et al., 1988). The 48-h LC$_{50}$ of PYM, reported as 7.5 mg L$^{-1}$ for P. reticulata and 5.9 mg L$^{-1}$ for O. mykiss (Canton and Van Esch, 1976), was 5.6 mg L$^{-1}$ in the present study.

Anton and Ariz (1994) reported that the 96-h LC$_{50}$ of TRC was 48.36 mg L$^{-1}$ for Carassius auratus and 92.72 mg L$^{-1}$ for Cyprinus carpio. LC$_{50}$ values were much lower for Anguilla anguilla, with 5.00 mg L$^{-1}$ at 24 h, 3.80 mg L$^{-1}$ at 48 h, 3.42 mg L$^{-1}$ at 72 h, and 3.38 mg L$^{-1}$ at 96 h (Ferrando et al., 1991). In the present study the 96-h LC$_{50}$ was 17.62 mg L$^{-1}$ for O. latipes, meaning that O. latipes was more susceptible to TRC than was A. anguilla. The higher the pH in water, the greater has been the amount of metabolite DDVP reported (Holmstedt et al., 1978; Chapman and Cole, 1982). The instability of TRC and its changing to DDVP also were observed in this study. Higher toxicity of DDVP compared to TRC has been demonstrated not only in mammals but in aquatic organisms (Holmstedt et al., 1978; Vaal et al., 1997). The trend of LC$_{50}$ values for O. latipes that were lower in the static test than in the semistatic test, seen at a late stage of the observation period in this study, may have been associated with longer exposure to DDVP.

The high sensitivity of TRC to D. magna had already been mentioned by the early 1970s (Zamfir et al., 1973). In the present study the 48-h EC$_{50}$ for D. magna was as low as 0.00026 mg L$^{-1}$. TRC is not only used as endo- and ectoparasiticides in farm animals but also is added to water to be exposed to Cypriniformes and Anguilliformes to control fish lice and anchor worms in aquaculture at concentration levels of 0.2–0.3 g ton$^{-1}$ of water. This water might be toxic to D. magna when released into the environment, even when diluted 1,000-fold, having adverse effects on an aquatic environment if discharged into rivers or streams without conventional wastewater processing. According to Espigares et al. (1997), TRC was detected at mean concentrations up to 0.00015 mg L$^{-1}$ in river water samples downstream from sewage treatment plants.

### TABLE II. Acute toxicity values of antiparasitic drugs in Oryzias latipes

<table>
<thead>
<tr>
<th>Compound</th>
<th>24 h LC$_{50}$ (mg L$^{-1}$)</th>
<th>95% Confidence Limit (mg L$^{-1}$)</th>
<th>48 h LC$_{50}$ (mg L$^{-1}$)</th>
<th>95% Confidence Limit (mg L$^{-1}$)</th>
<th>96 h LC$_{50}$ (mg L$^{-1}$)</th>
<th>95% Confidence Limit (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amprolium hydrochloride</td>
<td>&gt;600</td>
<td>NO</td>
<td>&gt;600</td>
<td>NO</td>
<td>&gt;600</td>
<td>NO</td>
</tr>
<tr>
<td>Bithionol$^*$</td>
<td>0.29</td>
<td>0.27–0.31</td>
<td>0.26</td>
<td>0.24–0.27</td>
<td>0.24</td>
<td>0.23–0.26</td>
</tr>
<tr>
<td>Levamisole hydrochloride</td>
<td>45.3</td>
<td>33.4–43.4</td>
<td>39.2</td>
<td>37.7–40.8</td>
<td>38.3</td>
<td>36.8–39.9</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>5.6</td>
<td>5.5–5.8</td>
<td>5.6</td>
<td>5.5–5.7</td>
<td>5.6</td>
<td>5.4–5.7</td>
</tr>
<tr>
<td>Trichlorfon</td>
<td>92.0</td>
<td>78.4–104.9</td>
<td>45.2</td>
<td>39.7–51.5</td>
<td>29.5</td>
<td>25.2–34.5</td>
</tr>
<tr>
<td>Trichlorfon$^*$</td>
<td>98.0</td>
<td>85.9–122.1</td>
<td>60.2</td>
<td>52.6–89.0</td>
<td>40.9</td>
<td>35.5–47.2</td>
</tr>
</tbody>
</table>

ND, not obtainable.

$^*$ Test was performed in semistatic condition.

### TABLE III. Acute toxicity values of antiparasitic drugs in Daphnia magna

<table>
<thead>
<tr>
<th>Compound</th>
<th>24 h EC$_{50}$ (mg L$^{-1}$)</th>
<th>95% Confidence Limit (mg L$^{-1}$)</th>
<th>48 h EC$_{50}$ (mg L$^{-1}$)</th>
<th>95% Confidence Limit (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amprolium hydrochloride</td>
<td>333</td>
<td>302–366</td>
<td>227</td>
<td>210–244</td>
</tr>
<tr>
<td>Bithionol</td>
<td>0.39</td>
<td>0.36–0.44</td>
<td>0.30</td>
<td>0.27–0.33</td>
</tr>
<tr>
<td>Levamisole hydrochloride</td>
<td>71.2</td>
<td>68.4–91.8</td>
<td>64.0</td>
<td>54.6–75.0</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>10.1</td>
<td>8.6–11.9</td>
<td>5.2</td>
<td>4.7–5.7</td>
</tr>
<tr>
<td>Trichlorfon</td>
<td>0.00052</td>
<td>0.00048–0.00057</td>
<td>0.00026</td>
<td>0.00023–0.00028</td>
</tr>
</tbody>
</table>
In the present study BT was shown to be more toxic than LVH and PYM to aquatic organisms. BT exhibited higher toxicity to *B. calyciflorus* than to *O. latipes* and *D. magna*. TRC was extremely toxic to *D. magna*. Taking into account the lowest LC50 or EC50 among the aquatic organisms in relation to the toxicity rating suggested by Constable et al. (2002), APH is categorized into a practically nontoxic class (>100 mg L\(^{-1}\)), LVH into a slightly toxic class (10–100 mg L\(^{-1}\)), PYM into a moderately toxic class (1–10 mg L\(^{-1}\)), and BT and TRC into an extremely toxic class (≤0.1 mg L\(^{-1}\)). In mammals, however, the acute oral LD50 values in mice were stated as 210 mg kg\(^{-1}\) for LVH and 408 mg kg\(^{-1}\) for BT, LVH being more toxic than BT (Yoshimura et al., 1985; Milne, 2002). The acute oral LD50 of TRC in rats was as high as 400–700 mg kg\(^{-1}\) (Holmstedt et al., 1978). In the oral rodent LD50 toxicity rating, BT and LVH are categorized into a very toxic class (50–500 mg kg\(^{-1}\)) and TRC into a very toxic or a moderately toxic class (500–5000 mg kg\(^{-1}\); Constable et al., 2002). A comparison with the the LD50s in rodents shows that the toxicity of antiparasitic drugs to aquatic organisms is likely not correlated with that to mammals, suggesting that antiparasitic drugs may exhibit unexpectedly higher toxicity to aquatic organisms when released into the environment.

In Japan cultivated land covers 480.3 million ha, of which 54.7% was paddy fields in 2000 (Statistics and Information Department, 2001). Given this regional peculiarity, aquatic organisms may have a greater chance of being exposed to antiparasitic drugs in Japan than in other countries, where agriculture depends mainly on farmland. This is because antiparasitic drugs may run off from the soil of a field spread with compost containing antiparasitic drug residues, dispersing into water in the rice paddies, regardless of their low solubility or insolubility in water. The greater the advance of decomposition of TRC in water, the higher is the toxicity to aquatic organisms by increasing the amount of the degradation product DDVP. BT is unstable in water, but a degradation product of BT is considered less toxic to aquatic organisms than BT itself because, unlike TRC, the LC50s of BT for *O. latipes* hardly changed throughout the observation period. However, possible adverse effects of BT on the environment should not be neglected in terms of high toxicity to aquatic organisms. LVH and PYM shown to be stable in water were confirmed to be slightly or moderately toxic to aquatic organisms. Although APH is considered practically nontoxic, an assessment of the hazard of even this compound to aquatic organisms still remains unknown (Boxall et al., 2003). Some medicinal components have been found in rivers and streams after discharge into the environment (Richardson and Bowron, 1985; Ternes, 1998). It is known that the antiparasitic drugs tested in this study are excreted more or less intact in feces and urine after administration to farm animals or in runoff from the back of the treated animals (Godfrain et al., 1969; Shimoda et al., 1981; Galtier et al., 1983; Hamamoto et al., 2000; Sahagún et al., 2001; WHO, 2001). The fate of antiparasitic drugs if they reach surface waters, especially from the soil of paddy fields, and whether their concentrations could be lethal to aquatic organisms need to be investigated in the future.

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### REFERENCES


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