



Original Article

## Assessment of the acute toxicity of 16 veterinary drugs and a disinfectant to aquatic and soil organisms

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**ABSTRACT** — In this study, we evaluated the toxicity of 16 veterinary drugs approved in Japan (anti-microbial substances, antiparasitic agents, and disinfectants) using a fish acute toxicity test, *Daphnia* swimming inhibition test, algae growth inhibition test, and earthworm acute toxicity test. Test organisms included *Oryzias latipes*, *Daphnia magna*, *Pseudokirchneriella subcapitata*, and *Eisenia fetida*. The tests were performed in accordance with the Organisation for Economic Co-operation and Development guidelines for testing chemicals. In the Globally Harmonized System of Classification and Labelling of Chemicals, when the EC/LC<sub>50</sub> value of a substance is < 1 mg/mL, then the substance is classified as Category 1, or highly toxic to aquatic organisms. The results of the present study indicate that the toxicity of tylosin phosphate, lincomycin, oxytetracycline chloride, and chlortetracycline to algae; that of ivermectin to crustaceans and fish; and that of didecyldimethylammonium chloride to all organisms examined should be classified as Category 1. In terrestrial organism, weak toxicity was observed with ivermectin, fenbendazole, and didecyldimethylammonium chloride. These data are considered to be valuable data for actually knowing the toxicity of veterinary drugs, in particular, since there is hardly any data following the guidelines using *Eisenia fetida*. Our results also suggest that the environmental risk of the tested veterinary drugs might be low at present researched environmental levels in river. In addition, our data indicate that the possibility of many veterinary drugs adversely affecting the environment is low if they are properly used.

**Key words:** Veterinary drugs, acute toxicity, *Oryzias latipes*, *Daphnia magna*, *Pseudokirchneriella subcapitata*, *Eisenia fetida*

### INTRODUCTION

Recently, concerns have increased over the presence of drugs in the aquatic environment. In Japan, the concentrations of drugs in aquatic environments range from undetectable to the order of several hundred nanograms per liter (Ishii *et al.*, 2004; Seino *et al.*, 2004; Yasojima *et al.*, 2004). Pharmaceutical products are available for both human and veterinary use. Because many drugs con-

tain the same active ingredient, the source of a particular drug in the aquatic environment cannot be determined. In a previous study, components of drugs used to treat pigs were detected in river basins of areas in which there were several pig farms, suggesting the possibility veterinary drugs can flow into rivers (Managaki *et al.*, 2007).

Veterinary drugs are medicinal products used exclusively in animals. In Japan, these drugs are approved by the Ministry of Agriculture, Forestry, and Fisheries

according to the “Act on Securing the Quality, Efficacy, and Safety of Products Including Pharmaceuticals and Medical Devices” and then manufactured and distributed. Veterinary drugs are approved when they are found to be effective and safe for target animals, of good quality, and safe for humans. However, the risks of these drugs to the environment are not subject to legal review. However, veterinary drugs can affect the environment even when properly used.

Increasing research has focused on environmental pollution due to pharmaceuticals. Many reports on the behavior of drugs in the environment and analytical methods for their detection have been published (Ishii *et al.*, 2004; Managaki *et al.*, 2007; Seino *et al.*, 2004; Yasojima *et al.*, 2004). Nevertheless, those drugs capable of adversely affecting ecosystems have not been identified. Currently, environmental impact assessment of new veterinary drugs is stipulated in the International Conference on Harmonisation of Technical Requirement for Registration of Veterinary Medical Products (VICH) guidelines. As the VICH guidelines are applied to all new veterinary drugs, the environmental impact of these drugs is addressed. However, because the VICH guidelines are not applicable to existing veterinary drugs, their environmental impact remains unknown. Thus, little information is available regarding the environmental risks of a majority of veterinary drugs (Eguchi *et al.*, 2004; Fukunaga *et al.*, 2006; Garric *et al.*, 2007; Lützhøft *et al.*, 1999; Halling-Sørensen, 2000; Yang *et al.*, 2008), and reliable data is desired (Miller *et al.*, 2019).

In this study, we evaluated the environmental impact of various veterinary drugs used in Japan in order to establish appropriate reduction methods if their impact is significant. As the first step in this process, we assessed the acute toxicity of these drugs to aquatic organisms (algae, *Daphnia*, and fish) and soil organisms (earthworms) according to the Organisation for Economic Co-operation and Development (OECD) test guidelines and examined their degree of ecotoxicity risk.

## MATERIALS AND METHODS

### Veterinary drugs

We selected 16 veterinary drugs that are considered to be used in large amounts in Japan (Japan Veterinary Products Association, 2004). The concentration of these drugs released into the environment or their effect on environmental organisms is considered high. The veterinary drugs used in this study included nine antibiotics, namely benzylpenicillin (PCG), cefazolin (CEZ), dihydrostreptomycin (DSM), kanamycin (KM), oxytetra-

cycline chloride (OTC), chlortetracycline (CTC), lincomycin (LCM), tylosin phosphate (TS), and colistin sulfate (CS); three sulfonamides, namely sulfamonomethoxine (SMMX), sulfamethoxazole (SMX), and sulfadimethoxine (SDMX); three anthelmintic agents, namely fenbendazole (FBZ), ivermectin (IVM), and cyromazine (CM); and one disinfectant: didecyldimethylammonium chloride (DDAC). Information on abbreviations, classifications, CAS nos., purity, lot numbers, manufacturers, and distributors of the test drugs is presented in Table 1.

### Toxicity test procedures

#### *Fish acute toxicity test*

Fish acute toxicity tests were conducted using *Oryzias latipes* provided by Shonan-Aquarium (Kanagawa, Japan). We introduced *Oryzias latipes* from local providers where we are able to confirm the breeding situations directly. Fish were acclimated in the laboratory of the Research Institute for Animal Science in Biochemistry and Toxicology for at least 14 days. During the acclimation period, the fish were fed Tetra KilliMin (Spectrum Brands Japan, Kanagawa, Japan). Toxicity tests were conducted in accordance with the “OECD Test Guidelines for Testing of Chemicals, No. 203”. Briefly, 10 fish (body length: approximately  $2.0 \pm 0.5$  cm) were exposed to five different concentrations of each veterinary drug in a 3000 mL borosilicate glass beaker. The maximum drug concentration was 100 mg/L (concentration determined in preliminary tests), which was subjected to a maximum 2-fold serial dilution. Breeding water (aerated dechlorinated tap water) was used for dilutions, without auxiliary. Fish were not fed from 24 hr before the test until the end of the study. Half of the pharmaceutical solution was replaced every 24 hr. The concentration of drug sufficient to kill 50% of the test fish (lethal concentration [LC]<sub>50</sub>) was determined based on the number of dead fish observed after a 96 hr drug exposure.

#### *Daphnia sp. acute immobilization test*

The *Daphnia magna* immobilization test (DAPH-TOXIKIT F) was purchased from MicroBio Tests Inc. (Ghent, Belgium). Young daphnids within 90 hr of hatching were used for the test and given the feed included with the kit. Acute immobilization tests were conducted in conformity with the “OECD Test Guidelines for Testing of Chemicals, No. 202”. Five daphnids were exposed to five different concentrations of each veterinary drug in a 1000-mL borosilicate glass beaker. The maximum drug concentration was 100 mg/L (concentration determined in preliminary tests), which was subjected to maximum 2-fold serial dilution. Breeding water (aerated dechlorin-

## Acute toxicity testing of veterinary drugs

**Table 1.** List of tested veterinary drugs.

Veterinary drug	Abbreviation	Classification	CAS No.	Purity	Lot No.	Manufacturer/distributor
Benzylpenicillin	PCG	$\beta$ -lactam antibiotic	6130-64-9	98.3%	23924302	LKT Laboratories, Inc
Cefazolin	CEZ	$\beta$ -lactam antibiotic	27164-46-1	98.8%	U4V8D	Tokyo chemical industry Co., Ltd.
Dihydrostreptomycin	DSM	Aminoglycoside antibiotic	5490-27-7	770 $\mu$ g/mg	R21510	MP Biomedicals, LLC
Kanamycin	KM	Aminoglycoside antibiotic	25389-94-0	760 $\mu$ g/mg	D00040321	Calbiochem
Oxytetracycline chloride	OTC	Tetracycline antibiotic	79-57-2	89.2%	5006H	Wako pure chemical corporation
Chlortetracycline	CTC	Tetracycline antibiotic	64-72-2	97.3%	9462J	MP Biomedicals, LLC
Lincomycin	LCM	Lincomycin antibiotics	7179-49-9	100.4%	GJ01	Tokyo chemical industry Co., Ltd.
Tylosin phosphate	TS	Macrolideantibiotics	1405-53-4	97.0%	23924411	LKT Laboratories, Inc.
Colistin sulfate	CS	Peptide antibiotic	1264-72-8	98%	TRC-04040108	TRC, Inc.
Sulfamonomethoxine	SMMX	Sulfa drug	1120-83-3	100.3%	EWJ4792	Wako pure chemical corporation
Sulfamethoxazole	SMX	Sulfa drug	723-46-6	100.1%	DPE2532	Wako pure chemical corporation
Sulfadimethoxine	SDMX	Sulfa drug	122-11-2	99.85%	23930406	LKT Laboratories, Inc.
Fenbendazole	FBZ	Anthelmintic	43210-67-9	99.15%	23910004	LKT Laboratories, Inc.
Ivermectin	IVM	Anthelmintic (macrolide)	70288-86-7	96.8%	23925407	LKT Laboratories, Inc.
Cyromazine	CM	Anthelmintic	66215-27-8	99.3%	23925807	LKT Laboratories, Inc.
Didecyldimethylammonium chloride	DDAC*	Disinfectant	7173-51-5	10%**	CH7H70	Tamura Seiyaku corporation

\*: Used veterinary drug named Cleakil-100

\*\*: Content in product

ated tap water) was used for dilution, without auxiliary. During the tests, the water temperature was maintained at  $20 \pm 1^\circ\text{C}$ , with a 16-hr light/8-hr dark cycle. The test water was not exchanged or replaced during the tests. The exposure time was 48-hr. Immobilization was defined as the state in which daphnia exhibited no movement for 15 sec after gentle movement of the container. The concentration required to immobilize 50% of test daphnids (effective concentration  $[\text{EC}]_{50}$ ) was then determined.

#### Algal growth inhibition test

An algal growth inhibition test kit using *Pseudokirchneriella subcapitata* (ALGALTOXKIT F) was purchased from MicroBio Tests Inc. The tests were conducted in conformity with the “OECD Test Guideline for Testing of Chemicals, No. 201”. Briefly, suspensions of algae pre-incubated in borosilicate glass Erlenmeyer flasks with silicone stoppers were exposed to five different concen-

trations of the veterinary drugs in 300 mL of OECD-recommended medium ( $\text{NH}_4\text{Cl}$  15 mg,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  12 mg,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  18 mg,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  15 mg,  $\text{KH}_2\text{PO}_4$  1.6 mg,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  0.08 mg,  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  0.1 mg,  $\text{H}_3\text{BO}_3$  0.185 mg,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  0.415 mg,  $\text{ZnCl}_2$  0.003 mg,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  0.0015 mg,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  0.0001 mg,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  0.007 mg, and  $\text{NaHCO}_3$  50 mg dissolved in 1000 mL of ultrapure water, sterilized by filtration using 0.22- $\mu\text{m}$  pore size filters, with pH of approximately 8.0 confirmed before use.). Algae were exposed to test drugs at  $23^\circ\text{C}$  with illumination controlled at 5000 lux and shaking of the culture at 100 rpm (BR-3000LF, Titech Co., Ltd., Aichi, Japan). The initial algal density was  $1 \times 10^4$  cells/mL. The maximum drug concentration was 100 mg/L (concentration determined in preliminary tests), which was subjected to maximum 2-fold serial dilution. Ultrapure water was used for dilution, without auxiliary. The number of algae was determined every

24 hr during the 72 hr exposure period using a UV/visible spectrophotometer (V-660, JASCO Corp., Tokyo, Japan) at 670 nm after calibrating the algal counts.  $EC_{50}$  values were calculated by probit analysis. The no observed effect concentration (NOEC) was also determined for the selected pharmaceuticals.

#### *Earthworm acute toxicity test*

Earthworm acute toxicity tests were conducted using *Eisenia fetida* provided by Sagami Joka Service (Kanagawa, Japan). We introduced *Eisenia fetida* from local providers where we are able to confirm the breeding situations directly. Earthworms were acclimated in the laboratory of the Research Institute for Animal Science in Biochemistry and Toxicology for at least 7 days. The tests were conducted in conformity with the "OECD Test Guidelines for Testing of Chemicals, No. 207". Approximately 20% water (ion-exchanged water) based on dry weight was added to artificial soil comprised of 10% peat moss (Konan Syoji Co. Ltd., Osaka, Japan), 20% kaolin (Wako Pure Chemical Corporation, Osaka, Japan), and 70% quartz sand (30% Wako Pure Chemical Corp., 40% Merck Corp., Tokyo, Japan). Calcium carbonate was used to adjust the pH to 6.5. Briefly, 10 mature earthworms (total body weight: 300-600 g) were exposed to at least five different concentrations of the veterinary drugs in a 1000 mL borosilicate glass beaker. The maximum drug concentration was 1000 mg/L (concentration determined in preliminary tests), which was subjected to maximum 2-fold serial dilution. Ultrapure water was used for dilution, without auxiliary. The exposure time was 14 days, at which time the  $LC_{50}$  values were determined.

#### **Data analysis**

Acute toxicity levels of the veterinary drug compounds were calculated using EcoTox-Statics Ver. 2.6d software (which is freely downloadable from the Japanese Society of Environmental Toxicology) and Regression Analysis Soft StatLight (Yukms, Kanagawa, Japan). Fish 96-hr  $LC_{50}$ , *Daphnia* 48-hr  $EC_{50}$ , algal 96-hr  $EC_{50}$ , earthworm 14-day  $LC_{50}$  values, and the arithmetic mean of the NOEC were determined.

### **RESULTS**

According to the OECD Test Guideline, acute toxicity tests were performed using four species.

All tests were conducted after dose setting in preliminary tests. In fish (*O. latipes*) acute toxicity test, the drug was exposed for 96 hr (total water was replaced every 24 hr), the mortality (%) at each concentration was cal-

culated, and the  $LC_{50}$  at 96 hr was determined by the Probit method. In crustaceans (*D. magna*) acute immobilization test, the drug was exposed for 48 hr (without water replacement), the immobilization inhibition rate (%) at each concentration was calculated, and the  $EC_{50}$  at 48 hr was determined by the Probit method. Algal (*P. subcapitata*) growth inhibition test was exposed to the drug for 72 hr (without water replacement), the cell concentration at each concentration was calculated, the  $EC_{50}$  for 72 hr was calculated by the Probit method. Earthworm (*E. fetida*) acute toxicity test was exposed to the drug for 14 days, the mortality (%) at each concentration was calculated, and the  $LC_{50}$  at 14 days was determined by the Probit method.

#### **Effects on aquatic species**

The results of *O. latipes*, *D. magna*, *P. subcapitata*, and *E. fetida* are presented in Table 2. The tested veterinary drugs were classified as A: highly toxic to algae; B: highly toxic to crustaceans and fish; C: toxic to all aquatic species; and D: weakly toxic or not toxic to aquatic species.

##### *A. Drugs highly toxic to algae*

LCM, TS, OTC, and CTC were highly toxic ( $EC_{50} < 1$  mg/L) to algae. KM, DSM, SMX, SMMX, and SDMX were also toxic to algae ( $EC_{50}$  1-10 mg/L). However, these drugs exhibited low toxicity ( $EC_{50} > 100$  mg/mL) to crustaceans and fish.

##### *B. Drugs highly toxic primarily to crustaceans and fish*

IVM was highly toxic to crustaceans and fish, whereas FBZ was highly toxic to fish. FBZ did not affect algae and fish at the highest clinically applicable concentration.

##### *C. Drugs toxic to all aquatic species*

DDAC was highly toxic ( $EC_{50}/LC_{50} < 1$  mg/L) to green algae, crustaceans, and fish. CS was highly toxic to green algae and toxic to crustaceans and fish.

##### *D. Drugs weakly toxic or not toxic to aquatic species*

PCG was only weakly toxic ( $EC_{50}$  10-100 mg/L) to crustaceans. CEZ and CM exhibited minimal toxicity to the three aquatic species tested.

#### **Effect on earthworms**

The  $LC_{50}$  values of DDAC, IVM, and FBZ were 600, 409, and 83.5 mg/L, respectively. These drugs were found to be toxic to earthworms. The  $LC_{50}$  values of PCG, CEZ, DSM, KM, OTC, CTC, LCM, TS, CS, SMMX, SMMX, SDMX, and CM exceeded 1000 mg/L of the upper limit

## Acute toxicity testing of veterinary drugs

**Table 2.** Toxicity of the tested drugs against fish, *Daphnia*, algae, and earthworms.

	Fishes	Crustacean	Algae	Earthworms
	<i>Oryzias latipes</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Eisenia fetida</i>
	LC <sub>50</sub> (mg/L)	EC <sub>50</sub> (mg/L)	EC <sub>50</sub> (mg/L)	LC <sub>50</sub> (mg/L)
	96 hr	48 hr	72 hr	14 d
PCG	>100	51.9	>100	>1000
CEZ	>100	>100	>100	>1000
DSM	>100	>100	1.29	>1000
KM	>100	>100	6.95	>1000
OTC	>100	>100	0.5	>1000
CTC	58.8	>100	0.862	>1000
LCM	>100	>100	0.783	>1000
TS	>100	>100	0.503	>1000
CS	3.48	2.82	0.104	>1000
SMMX	>100	>100	8.48	>1000
SMX	>100	>100	1.85	>1000
SDMX	>100	>100	17.0	>1000
FBZ	>0.035*	0.0159	>0.35*	83.5
IVM	0.00540	40.0 ng/mL	>3.5*	409
CM	>100	>100	>100	>1000
DDAC	0.425	0.292	0.0443	600

\*: The maximum concentration that could be used for the test.

concentration in the test guideline; therefore, these drugs were judged to be not acutely toxic to earthworms.

Earthworms tended to come out of the soil and collect on the surface of veterinary drugs that were confirmed to be toxic.

### Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification criteria

Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification criteria

Based on the results obtained in this study, the fish 96-h LC<sub>50</sub>, *Daphnia* 48-h EC<sub>50</sub>, and algal 96-h EC<sub>50</sub> for each drug were plotted (Fig. 1). The drugs with an LC<sub>50</sub> or EC<sub>50</sub> value of ≤ 1 mg/L were classified as GHS category 1.

## DISCUSSION

In this study, we evaluated the environmental impact of a number of veterinary drugs commonly used in Japan in order to facilitate establishment of reduction methods for compounds exhibiting a high environmental impact. Testing of the acute toxicity of 16 veterinary drugs to aquatic and soil organisms was performed, and the degree of toxicity risk was determined.

Antibiotics such as aminoglycosides, tetracyclines, and macrolides, which inhibit bacterial protein synthe-

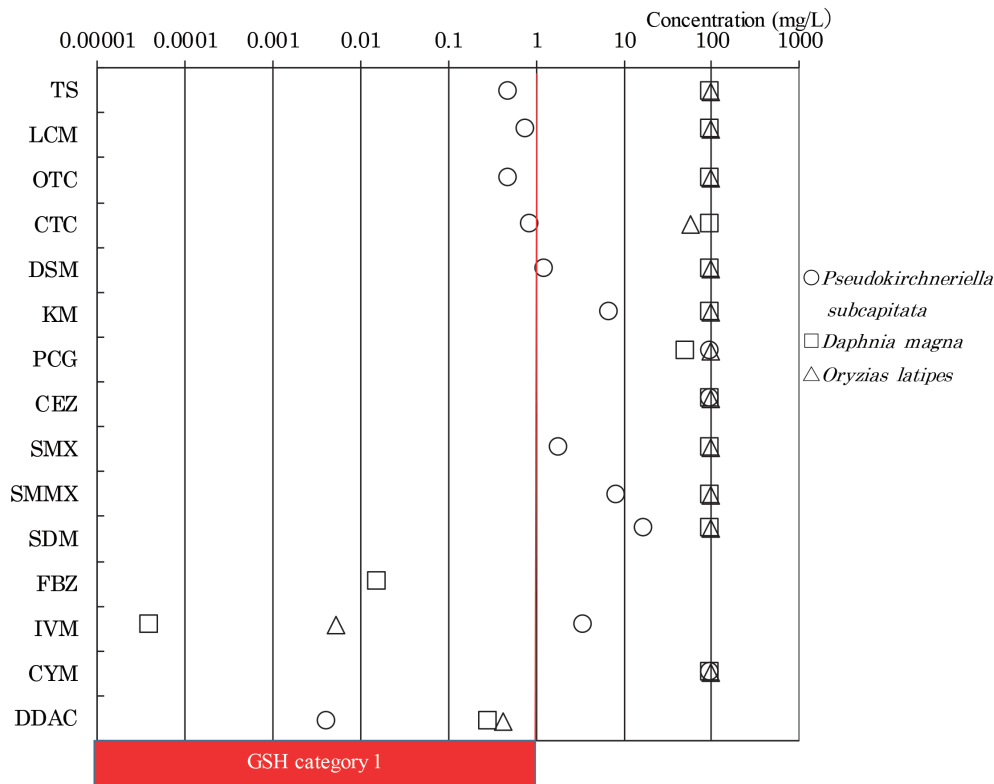
sis (Chopra and Roberts, 2001; Fernandes *et al.*, 2017; Mingeot-Leclercq *et al.*, 1999; Szezuka *et al.*, 2016), and sulfonamides, which inhibit bacterial folic acid synthesis (Sadaka *et al.*, 2018), are veterinary drugs that are reportedly highly toxic to aquatic organisms, primarily algae. Antibiotics that inhibit protein synthesis do so by binding to and disrupting the function of the bacterial ribosomal subunit. The function of chloroplasts of single-cell green algae is thought to be inhibited by these drugs due to their structural similarity to bacterial ribosomes (Sharma *et al.*, 2007). As the ribosomal subunits of *Daphnia* and fish have a eukaryotic structure that differs from that of bacterial ribosomes, it is assumed that the toxic effects of antibiotics are not manifested in these organisms.

As the folic acid synthesis pathway is also present in plants, sulfonamides exhibited toxicity to algae. However, other aquatic organisms (crustaceans and fish) did not exhibit signs of toxic effects due to the absence of a pathway for folate synthesis in animal cells.

IVM inhibits glutamate-gated chloride channels in parasites (Laing *et al.*, 2015), and FBZ inhibits intracellular microtubule formation (Duan *et al.*, 2013; Spagnuolo *et al.*, 2010). Because both crustaceans and fish have glutamate-gated chloride channels that form intracellular microtubules, IVM and FBZ exhibited strong toxic effects against these organisms.

Drugs that affected all aquatic species included CS,





**Fig. 1.** Veterinary drugs belonging to GHS classification criteria acute category 1 (<1.0 mg/L)

which acts on membrane phospholipids with antibiotics and demonstrates plasma membrane degenerative effects (Rhouma *et al.*, 2016), and DDAC, which exhibits cationic surfactant activity with disinfectants (Yoshimatsu *et al.*, 2007). CS and DDAC were found to be toxic to all tested species due to their plasma membrane-denaturing and physicochemical actions.

PCG and CEZ, which inhibit bacterial cell wall synthesis (Drawz and Bonomo, 2010), were not toxic to algae, crustaceans, and fish because these organisms do not have targets for these drugs. The toxicity values of PCG and CEZ were similar to those reported in other studies using the same organisms in acute toxicity tests (Garrie *et al.*, 2007; Hally *et al.*, 1989; Yang *et al.*, 2008).

IVM, FBZ, and DDAC exhibited toxic effects against earthworms. These toxic effects were due to IVM-mediated inhibition of glutamate-gated chloride channels, FBZ-mediated inhibition of intracellular microtubule formation, and the surfactant-mediated physicochemical activity of DDAC. However, antibiotics and sulfa drugs were not toxic to earthworms.

For aquatic organism toxicity tests, water conversion was performed according to OECD Test Guidelines. The

drug concentration in water decreased with the DDAC and tetracyclines over time. Since DDAC is a surfactant, it was thought the concentration in the water was lowered by adsorption to container, organisms, and the medium contained in the water. DDAC had the highest decay rate, and in algal tests, it was less than 0.0005 mg/L at all test concentrations 3 hr after the start of the test. For tetracyclines, OTC and CTCs are known to be degraded in water, and drug concentrations decreased throughout the study. On the other hand, sulfa drugs and macrolides did not decrease after 24 or 72 hr.

Migrate of veterinary drugs to earthworms was measured only for IVM. However, although there is considerable variation among individuals and the clear migration is unknown, the migration to earthworms has been confirmed, including individuals who survived the test period. The data of acute toxicity tests conducted according to the guidelines using *E. foetida* are scarce to the best of our knowledge, so the data of 16 different veterinary drugs are considered valuable.

Based on the results of this study, the veterinary drugs that exhibited acute toxicity to algae, crustaceans, and fish were classified as acute category 1 (very toxic to aquatic

life) based on the GHS classification criteria for aquatic organisms (Fig. 1). Some veterinary drugs exhibited low toxicity to algae, whereas most drugs were not toxic to *Daphnia* and fish. Among the agents tested against earthworms, FBZ was the most toxic, followed by IVM. Both FBZ and IVM are anthelmintic agents that are toxic to soil organisms.

An analysis of reported drug concentrations in rivers suggested that they are not immediately problematic (Managaki *et al.*, 2007). The present results are considered to pose a low risk at this time, but if there is an abnormality in the treatment of waste water or waste, there is a concern that the environmental concentration will rise temporarily, affecting the environmental organisms. Furthermore, used veterinary drugs excreted into manure should be released not only to rivers but also to the soil through compost. There must be the possibility of local accumulation of veterinary drugs in soil by using composts containing veterinary drugs. At present, acute toxicity was observed in some veterinary drugs in earthworm, a terrestrial organism. Therefore, it is important to encourage prudent use of veterinary drugs.

In conclusion, we conducted fish acute toxicity, *Daphnia* acute immobilization, algal growth inhibition, and earthworm acute toxicity tests for 16 veterinary drugs. High toxicity, with an  $EC_{50}$  or  $LC_{50}$  value of  $\leq 1$  mg/L, was observed for DDAC in fish; DDAC, IVM, and FBZ in *Daphnia*; and DDAC, OTC, CTC, LCM, TS, and CS in algae. This organism-specific toxicity could be explained by the pharmacologic properties of the tested drugs. This is the first report to comprehensively investigate the toxicity of veterinary drugs to environmental organisms. There are few data according to the guidelines for acute toxicity tests of terrestrial organisms, especially earthworms. Although the concentrations of the tested drugs detected in Japanese rivers are currently considered to pose a low risk. Our findings indicate that the possibility of veterinary drugs adversely affecting the environment is low if they are properly used. It is necessary to reduce the use of veterinary drugs and their concentrations in wastewater to maintain this level of low environmental risk.

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**Conflict of interest----** The authors declare that there is

no conflict of interest.

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