Effects of the insecticide esfenvalerate on zooplankton in an indoor synthetic model ecosystem

Mitsugu Miyamoto* and Takuo Fujisawa

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 2–1, Takatsukasa 4-chome, Takarazuka, Hyogo 665–8555, Japan

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Effects of esfenvalerate on zooplankton and their recovery potential were studied using an indoor synthetic model ecosystem. Esfenvalerate was applied to the system by direct spiking to overlying water or the introduction of treated slurry soil to imitate drift and run-off exposure. Four zooplankton taxa, *Daphnia magna* (Cladocera), *Heterocypris incongruens* (Ostracoda), *Cyclops* sp. (Copepoda), and *Brachionus calyciflorus* (Rotifera), were exposed to esfenvalerate with *Raphidocelis subcapitata* (green alga).

In a drift scenario, lower doses (0.02–0.5 µg/L) showed slight or negligible effects, but the results for the highest dose (5 µg/L) indicated direct effects, as remarkable population decreases were observed for three taxa (except rotifera). However, for the latter dose, results of weekly or single (Day-15) zooplankton re-introductions after dosing demonstrated resilient recovery. In a run-off scenario, a nominal dose of 20 µg/L, in which the measured water concentrations remained at 3–6%, had effects similar to those of the high drift exposure scenario for cladocera and ostracoda.

Keywords: synthetic model ecosystem, indoor microcosm, zooplankton, esfenvalerate.

Introduction

Aquatic multispecies model ecosystem, so-called microcosm/mesocosm, experiments have been widely used as higher-tier testing tools for ecological risk assessments of chemicals, especially pesticides, in the EU. Indoor microcosm dominated by zooplankton established from natural pond water and sediment with associated organisms have often been used as more practical alternatives to outdoor ones. However, even in these indoor microcosms, limitations on the representativeness and reproducibility of available taxa remain; thus, further improvements or supplemental tools are considered necessary. In this respect, indoor standardized or synthetic microcosms that are constructed using artificial inoculations of cultured species can be among the resolutions. Taub et al. and other researchers have investigated these systems and contributed to the development of related guidelines such as ASTM and US EPA OPPTS. However, in spite of these achievements, the standardization of zooplankton taxa has been limited to *Daphnia magna* representing Cladocera, and others such as Ostracoda, Rotifer, and Copepoda have been omitted or treated rather ambiguously, which might result in great variability among the characteristics of established plankton communities. In addition, most of the above-mentioned experiments/guidelines adopted an artificial sediment simply made from silica sand and a carbon source (chitin and cellulose), which may not adequately reflect the chemical behaviors observed in the natural environment.

In the meantime, modern ecotoxicological testing tools have been improved, such as the supply of reference species by a public reference laboratory, e.g., The National Institute for Environmental Studies (Ibaraki, Japan), and standard sediment recipes developed by various research organizations. In fact, several zooplankton, including ostracoda and rotifera species, can be purchased and an artificial OECD sediment for midge testing, consisting of sand, kaolin clay, and peat has been widely used and can be conveniently applied to refine the standardization and reproducibility of indoor synthetic microcosm experiments. In addition, the aquatic exposure scenarios (e.g., pond, river, ditch) and main entry routes (e.g., drift, drainage, run-off) of pesticides for the ecological risk assessment differ greatly, depending on the use patterns and pesticidal properties, as well as regulatory authorities. Moreover, the populational recovery of affected taxa via survivors in refugia or immigration inflow is considered to be one of the key factors for higher-tier risk assessment. Therefore, the accumulation of modification tools for the microcosms to reflect various conditions such as those mentioned above, is deemed inevitable.

From these viewpoints, we have established an indoor synthetic plankton community microcosm consisting of four typical zooplankton taxa with a phytoplankton. In addition, its usefulness, such as by the modification of populations to estimate re-
covery potential and a comparison of ecotoxicological differences between simulated run-off and drift exposure, was investigated using a pyrethroid insecticide, esfenvalerate (Sumi-Alpha®, SMA) [(αS)-α-cyano-3-phenoxybenzyl (2S)-2-(4-chlorophenyl)-3-methylbutyrate], which shows high toxic potential to zooplankton (ppb level or below) and has much outdoor mesocosm data.

4. Materials and Methods

1. Chemicals

SMA (purity 98.0%) was purchased from Kanto Chemical Co., Inc. (Japan) and used as the test substance and analytical standard. Additionally, two analytical standards for possible degradation products, 3-Phenoxybenzoic acid (PBacid) and 2-(4-Chlorophenyl)isovaleric acid (CPIA), with purities ≥98.4%, were prepared in our laboratory. Other chemicals were of a reagent grade and purchased from commercial suppliers, unless otherwise noted.

2. Test organisms

Four kinds of zooplankton, Daphnia magna, Heterocyclops incongruens, Cyclops sp., and Brachionus calyciflorus, which representing typical zooplankton taxa for Cladocera, Ostracoda, Copepoda, and Rotifers, respectively, were multigenerationally cultivated in our laboratory. Their parents were originally obtained from Sumika Technoservice Corporation (Japan) for D. magna, and from MicroBioTests Inc. (Belgium) for H. incongruens and B. calyciflorus as cysts. The parental copepod was collected from a pond (34.778N, 135.483E) in Osaka Prefecture in Japan. They were cultured under controlled conditions, namely 4–8 L vessels filled with dechlorinated tap water in a temperature-controlled water bath (20±1°C) under a 16-hr daylight photoperiod, and regularly fed with commercially available chlorella (Chlorella V12, Chlorella Industry Co., Ltd., Japan).

A representative phytoplanktonic green alga, Raphidocelis subcapitata (formerly known as Selenastrum capricornutum or Pseudokirchneriella subcapitata), was originally obtained from Sumika Technoservice Corporation. Precultures of R. subcapitata suspension solutions of >10⁶ cells/mL were prepared prior to each bioassay from stock in a refrigerator and incubated at 23–26°C in media as referred to in OECD guideline 201 under continuous shaking and illumination by fluorescent bulbs.

3. Water, sediment, and soil

Tap water dechlorinated with activated charcoal was used for culturing zooplankton and the overlying water of the model ecosystem. Artificial sediment as described in OECD guideline 218, consisting of approximately 75% sand, 20% kaolin clay, 3% peat (Protoleaf Inc., Japan), and 0.3% CaCO₃, was employed for the system. Clay loam soil (sand: 55%, silt: 24%, clay: 21%, organic carbon: 2.0%; pH: 5.2; moisture: ca. 13%) collected in early spring from the top 10 cm of an orchard farm field in Kasai (Hyogo Prefecture, Japan) with no pesticide treatment for more than three years was used as substrate soil to prepare simulated run-off slurry. The soil was passed through a 2 mm mesh sieve to remove stones and plant debris prior to use without any other treatment (e.g., sterilization, drying).

4. Model ecosystem bioassays

4.1. Test system setup, management, and observation

Indoor plankton model ecosystems were constructed in 5 L (16×21×23 cm) glass vessels immersed in a temperature-controlled water bath (20±1°C) under 16-hr daily lighting by fluorescent bulbs with supplemental 10 hr high illumination (ca. 18,000–20,000 lux) by 400 W metal halide lamps (National, Japan) on the ceiling. In the vessels, the model ecosystems were prepared using 2 cm depth of OECD 218 artificial sediment and 5 L of dechlorinated tap water with mild aeration (several bubbles/sec) at mid-depth along the vessel wall for water quality maintenance (support of convection flow and the prevention of biofilm and oxygen deficiency), and a weekly addition of an N/P source (150 ng of NaNO₃ and 90 ng of K₂HPO₄) for algal growth. At the initiation of the biological setup of a model ecosystem, 1–10 mL suspension solutions of R. subcapitata (>10⁶ cells/mL) and B. calyciflorus (ca. 15–18 individuals/mL), 20–30 subadults of D. magna, 10–40 subadults of H. incongruens, and 10–20 subadults of Cyclops sp. were introduced into each vessel and acclimated for more than four weeks to establish stable and mature plankton populations prior to the test material dosing.

Water quality and plankton populations were monitored weekly. Water temperature, pH, and dissolved oxygen (DO) were measured using an alcohol thermometer or multi-thermometer (Japan Pet Drugs Co., Ltd., Japan), pH meter model B-212 (Horiba Ltd., Japan), and DO meter SevenGo pro (Mettler Toledo, USA) or model DO-5509 (FUSO Co., Ltd., Japan), respectively. Depth-integrated water samples (ca. 500 mL) were temporarily obtained by means of a tube sampler at several positions of the vessel after stirring the overlying water very gently for the visual counting of D. magna, H. incongruens, and Cyclops sp.; in addition, a 50 mL sample was further divided and subjected to the counting of copepod nauplii and B. calyciflorus under an optical microscope (model SZX16, OLYMPUS, Japan). The water samples were returned to the test vessel after the observations. As a surrogate of the algal population, the chlorophyll-a concentration was measured using a chlorophyll and turbidity sensor (Infinity-CLW, JFE Advantech Co., Ltd., Japan).

4.2. Exposure experiments

4.2.1. Simulated drift pulsed-dose experiment

After acclimation, 11 microcosm vessels were assigned as three untreated controls and eight vessels treated with SMA at four concentrations in duplicate (0.02, 0.1, 0.5, and 5 μg/L). A series of SMA stock solutions was prepared using acetone, and the overlying water in each test vessel was spiked once with the corresponding stock solution (0.1 mL/L), followed by gentle mixing with a spatula to prepare uniform exposure concentrations. The model ecosystems were monitored for eight weeks after SMA treatment.

4.2.2. Simulated extrinsic immigration recovery experiment
In parallel to the above-mentioned pulsed-dose experiment, six model ecosystems were set up, maintained, and allocated to imitate two immigration scenarios as a re-introduction of zooplankton equivalent to “ca. 5% population of control group” and “ca. 100% population of control group” as surrogate examples to reflect periodical small-scale immigration inflow and a single large-scale immigration one, respectively. Namely, in the former case, a small portion of zooplankton (subadults of 20 D. magna, two H. incongruens, two Cyclops sp., and five B. calyciflorus) was added on a weekly basis after SMA dosing (Day-0). Two different groups of vessels, i.e., SMA treatment at 5 µg/L and an untreated control, were assigned in duplicate. For the latter group, the mass introduction of SMA-sensitive taxa (approximately 350 of D. magna, 56 of H. incongruens, and 32 of Cyclops sp., randomly mixed population of juvenile-adults from a portion of original stock cultures, the population levels approximately equivalent to the corresponding untreated control) was implemented once on Day-15, to confirm whether the water column was detoxicated over time. Two vessels treated with 5 µg SMA/L were assigned for the experiment.

4.2.3. Simulated run-off slurry introduction experiment

After acclimation, 15 ecosystem vessels were used for the experiment. On the day of treatment (Day-0), seven vessels received ca. 1.13 g of natural soil (1 g dry weight) as 50 mL of simulated run-off slurry, i.e., untreated soil was applied in three ecosystems and SMA mixed soil in the remaining four ecosystems at 10 ppm (0.01 mg SMA, nominal 2 µg/L in a 5-L water column) or 100 ppm (0.1 mg SMA, nominal 20 µg/L), in duplicate. In addition, the other eight vessels received ca. 11.3 g of natural soil as 50 mL of soil slurry, i.e., three treatment groups were made with untreated soil in quadruplicate, and 1 ppm SMA soil (0.01 mg SMA, nominal 2 µg SMA/L) and 10 ppm SMA soil (0.1 mg SMA, nominal 20 µg SMA/L) in duplicate. The slurry-treated ecosystems were monitored for seven weeks.

One day before the slurry introduction, SMA stock solutions were applied to the soil and mixed well to prepare 1, 10, and 100 mg/kg spiked soil samples and kept under ambient dark conditions overnight.

5. Chemical analysis

Measurements of SMA concentrations in the overlying water (depth-integrated sample) in representative microcosms were carried out regularly (2 hr to 14 days after treatment) using GC-ECD chromatography with a Shimadzu GC-2010 equipped with a DB-17HT (0.15 µm) fused silica capillary column (0.25 mm i.d. × 30 m). The helium carrier gas and nitrogen make-up gas flows were 2.9 and 60 mL/min, respectively. An oven temperature program of 200°C (1 min)–10°C/min–280°C (held for 3 min) with detector temperature of 320°C resulted in a typical SMA retention time of approximately 10.6 min with good linearity (r²=0.98, 0.1–50 µg/L standards).

Prior to the GC-ECD analysis, microcosm water samples of approximately 10 mL were extracted twice with n-hexane (7–8 mL), and the extracts were dried with Na₂SO₄ and subjected to Sep-Pak Silica cartridge clean-up (rinsed with 10 mL of n-hexane followed by 10 mL of n-hexane/ethyl acetate [1:10/1, v/v] elution). The eluate was evaporated to dryness and reconstituted with acetone for GC-ECD analysis (78–125% recoveries at 0.05–5 µg/L fortifications in clear or soil (fine particle) suspension water).

In addition, two possible degradation products, PBacid and CPIA, were analyzed in typical water samples from the simulated drift experiment. A Waters LC-MS system, consisting of a Micromass ZQ spectrometer equipped with a Waters Separation Module 2695 as a liquid chromatograph and an ODS column (Symmetry, 3.5 µm, 3.0 mm × 50 mm; Waters), was operated at a flow rate of 0.4 mL/min under an isocratic condition (acetonitrile/0.05% formic acid in water=45/55, v/v). The quantification of each degrade was carried out by monitoring ions with m/z values of 213 ([M–H]–, tR=ca. 22 min) for PB acid and 211 ([M–H]–, tR=ca. 2.8 min) for CPIA in an electrospray ionization (ESI) negative mode with a cone voltage of 15 V in direct injection analysis (0.1–3 µg/L, typical r²=0.89–0.99).

6. Data analysis

The numbers of counted zooplankton were converted to densities by using sampled water volume. The mean population density among the replicates was plotted and analyzed visually from the graph.

Results

In all bioassays, the water quality parameters of temperature, pH, and DO, as well as chlorophyll-a concentrations were well maintained without SMA treatment-related changes as 19.9–20.5°C, 7.9–8.6, 8.7–10.0 ppm, and 7–18 µg chl.a/L, respectively.

1. Simulated drift pulsed-dose experiment

A good SMA concentration series was confirmed on the treated-

![Fig. 1. Relationships of measured vs. nominal concentrations of SMA in a water column of simulated drift and run-off treatments](image)
day (2 hr) samples, with an overall average of 92% of the nominal concentrations (Fig. 1), and in those having received a representative treatment (5 μg/L), concentrations decreased rapidly with time (Fig. 2). Both of the expected degradates, PBacid and CPIA, were observed at trace levels below the limit of quantification (0.1 μg/L) in water samples after 2–14 days for the 5 μg/L treatment group.

The population of *D. magna* showed decreases for two high doses just after SMA treatment as a two-week transient decrease at 0.5 μg/L and complete extinction at 5 μg/L (Fig. 3). Similar but clearly weaker effects were observed for *Cyclops* sp., with no effect at 0.5 μg/L, and still lower sensitivity for *H. incongruens* with approximately a one-week transient decrease even at the highest concentration (5 μg/L). In contrast, *B. calyciflorus* populations at the highest dose showed a clear population increase after SMA treatment.

2. *Simulated extrinsic immigration recovery experiment*

Plankton populations in the untreated control microcosm with weekly plankton additions were generally similar to those of the original untreated controls (Fig. 4) in consideration of biological variation (e.g., variations before SMA application). In SMA 5 μg/L treatment vessels with weekly zooplankton additions, although temporary population decreases were observed for *D. magna*, *Cyclops* sp., and *H. incongruens*, they steadily recovered to the control levels within three weeks. The population increase of *B. calyciflorus* in the SMA 5 μg/L exposure vessels was less pronounced as compared with non-immigrating cases (Figs. 3 and 4).

In the Day-15 re-introduction group, the decreased populations of *D. magna*, *Cyclops* sp., and *H. incongruens* were recovered and maintained at the control levels after the addition of plankton. The population of *B. calyciflorus* was positively affected (increase) after SMA treatment, but was suppressed, and returned to the control level two weeks after the re-introduction of SMA-sensitive zooplankton.

![Fig. 2. SMA dissipation from a microcosm water column after simulated drift treatment](image1)

![Fig. 3. Population changes of zooplankton in simulated drift pulsed-dose SMA treatment](image2)
3. Simulated run-off slurry introduction experiment

The concentrations of SMA in sampled water on the day of treatment were determined to be 0.13–0.14 µg/L in lower-loading groups (nominal 2 µg/L: 10 ppm SMA × 1 g soil and 1 ppm SMA × 10 g soil) and 0.52–1.1 µg/L in higher-loading groups (nominal 20 µg/L: 100 ppm SMA × 1 g soil and 10 ppm SMA × 10 g soil), indicating significantly lower recovery from the water phase in contrast to those in the drift-simulated treatment groups (Fig. 1). The amount of SMA in the water phase was estimated to be 7% and 3–6% of the applied dose, respectively, for nominal 2 and 20 µg/L treatment groups.

*Cyclops* sp. and *B. calyciflorus* were threatened with extinction even in untreated control microcosms without clear causality, which caused some difficulty in verifying the effects of SMA treatment, but other plankton populations were generally abundant and stable after maturation (Fig. 5). For SMA-treated microcosms, the abundance of *D. magna* had clearly decreased after treatment, with intrinsic population recovery within five weeks in two higher-loading groups (20 µg/L), and slight or negligible changes in two lower-loading groups (2 µg/L). Populations of *H. incongruens* indicated slight or negligible changes, even in higher-loading groups.

**Discussion**

1. Behavior of SMA in a model ecosystem

In the simulated drift experiment, the observed rapid dissipation of SMA from the water column was in good agreement with that in available outdoor microcosm/mesocosm studies. A set of kinetic analyses tentatively conducted using a computer program (CAKE, Version 3.3, Tessella) revealed that a hockey-stick model was the best-fitted regression, with a minimum $\chi^2$ value of 0.42 in comparison with three other models (Single-First-Order: 12, First-Order-Multi-Compartment: 3.9, Double-First-Order-in-Parallel: 0.50), and the resultant DT$_{50}$ value was 0.57 days, which was within the range of the reported outdoor DT$_{50}$, 0.14–1.3 days ($n$ = 14 in five studies, geometric mean $= 0.37$ days). The main driver of this rapid dissipation was considered to be adsorption to the sediment rather than degradation, not only because of the highly adsorptive character of SMA (mean soil organic carbon–water partitioning coefficient $[K_{oc}]$ value: 251,700), but also due to the limitations on the photolytic and microbial degradation potential of the synthetic microcosm, i.e., the light characteristics (metal halide lamp) and microbial sources (artificial water and sediment with microbes only from peat). This assumption was confirmed, as trace levels of PBacid and CPIA (<0.1 µg/L, equivalent to <5% of applied SMA) were detected in the overlying water of the system, while a significant amount of degradates (e.g., the detection of PBacid in water >20% of treated SMA) was detected in indoor illuminated or dark water-sediment degradation studies as well as outdoor studies. However, such lower degradation potential of the indoor microcosm as compared with outdoor studies would...
contribute to a conservative evaluation.

For the run-off experiment, SMA recoveries as low as 3–7% of the applied amount in the water column were within expectations, taking into account the highly adsorptive properties of SMA. The water concentrations tentatively expected based on the $K_{oc}$ and soil organic carbon (OC) (2.0%) with a water volume of 5 L were estimated to range from 0.2 (1 ppm × 10 g soil, 0.2 g OC) to 20 (100 ppm × 1 g soil, 0.02 g OC) µg/L, values higher than those measured (0.13–1.1 µg/L). This difference could be simply explained by the extremely low water solubility of SMA (e.g., <1 µg/L). In the meantime, the SMA treatment concentrations in soil (1–100 ppm) used for this experiment were much higher than the maximum estimated concentration in soil (0.0276 ppm) calculated from a typical potato use in the EU (15 g SMA/ha × 3 applications), which clearly suggested negligible potential effects on zooplankton from the run-off exposure from actual agricultural usage.

2. Direct and indirect effects and recovery potential

In the pulsed-dose experiment, the typical taxon sensitivity expressed as direct effects in terms of the extent and duration of the population at 0.5–5 µg/L decreased in the order of $D. magna$ (Cladocera) > Cyclops sp. (Copepoda) > $H. incongruens$ (Ostracoda) > $B. calyciflorus$ (Rotifera). These sensitivity trends (taxon order and effect concentration ranges) were basically in agreement with the available outdoor microcosm/mesocosm data, although some special responses derived from complexity of the community were observed in outdoor systems (e.g., unclear causal sequence, occasional decrease of specific sensitive species, etc.) (Fig. 6). The population increase of rotifera, which would be a typical indirect effect caused by the depletion of competitors (e.g., cladocera) and lower sensitivity to toxicants than those of others (cladocera, copepoda, and ostracoda), had been similarly confirmed in most of the SMA outdoor studies.

The intrinsic population recoveries of $D. magna$ (0.5 µg/L) and $H. incongruens$ (5 µg/L) in the simulated drift experiment and the extrinsic population recoveries of $D. magna$, Cyclops sp., and $H. incongruens$ even at 5 µg/L treatment in the simulated immigration experiment, were assumed to be achieved as a result of exquisite balance among the presence of sufficient survivors (i.e., the extent of toxicity), the dissipation of stressors from the media (SMA DT50 of 0.57 days), and excellence in reproductive manners (e.g., R-strategist, short lifecycle, parthenogenesis, etc.). Population recoveries in the simulated immigration experiment were considered to be a good explanation for what was observed in outdoor systems, including SMA cases (e.g., Fig. 6) as well as actual environments, because the active (i.e., diurnal and seasonal movements by swimming) and passive (e.g., drift by water flow) movements and resistant life stages (i.e., hatching from wintering eggs or cysts) of zooplankton should act as immigration factors of the taxa in the natural hydrosphere. In addition, several refugia for zooplankton might exist around macrophytes, stones, and debris to protect them from chemical
For the simulated run-off experiment, the initial SMA concentrations in the water column, including the adsorbed fraction with suspended matter, generally correlated with the total SMA application rate but not with SMA concentrations in the treated soil or the amount of the soil introduced as slurry. Although data on copepoda and rotifera populations were unfortunately insufficient for evaluation, other (cladocera and ostracoda) population responses were generally the same as those in the simulated drift experiment, based on the initially measured SMA concentrations in the overlying water (Fig. 5). This result also suggested that the large amount of SMA (93–97%) existing in the sediment and deposited soil particles had almost negligible effects on the zooplankton. In the meantime, the reason for the extinction of copepoda and rotifera in all test groups in the early phase was uncertain; however, it might be related to imbalances between unidentified nutrition, water quality, or interpopulation relationships during the acclimation period (immature population phase).

3. Utility of the synthetic model ecosystem

From the viewpoint of establishing more convenient test ecosystems as compared to outdoor microcosm/mesocosm, several researchers investigated zooplankton-dominated indoor microcosms, including synthetic systems of various sizes, ranging from approximately 3–600 L, constructed mainly from natural water and sediment with their associated indigenous plankton. In general, the stability of the ecosystem would be positively correlated to their sizes. However, although our synthetic 5 L microcosm was at the lower end of the range above, it visually showed sufficient stability and reproducibility of plankton populations to evaluate pesticide effects over a few months.

Ostracoda and rotifera are considered to be important taxa in a microcosm for precise community evaluation; however, lack of or poor abundance of ostracoda taxa has often been observed in comparison with other taxa (cladocera or rotifera) in indoor microcosms using natural zooplankton. In fact, rotifera frequently play a unique role in ecological adaptation via indirect population increase, as shown in Fig. 6. Moreover, no internationally acknowledged standard species for ecotoxicological studies are available for ostracoda and rotifera. In these respects, our result indicates H. incongruens (Ostracoda) and B. calyciflorus (Rotifera) could be surrogate typical species.

With regard to the particular modifications of the test design, run-off or recovery simulations, such as partial treatment or re-introduction, have been demonstrated mainly in larger outdoor systems in the past. We have successfully applied run-off treatment and re-introduction designs for recovery potential evaluation as modifications of a synthetic microcosm test. Additional data generation on the following would be considered valuable: 1) the effects and chemical behavior of low-adsorptive pesticides by run-off treatment, 2) the effects including populational recovery for persistent pesticides, 3) the effects on populational recovery with different plankton re-introduc-

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**Fig. 6. Effects of SMA on zooplankton taxa in outdoor microcosms/mesocosms and synthetic indoor model ecosystem**

| Test Concentration (µg/L) |
|--------------------------|
| 0.0001 |
| 0.001 |
| 0.01 |
| 0.1 |
| 1 |
| 10 |

**Table 1.**

| Effect class: No effect (○), Population decreased effect with recovery (●) or without recovery (■), Population increased effect with recovery (▲) or without recovery (▼); Microcosm/Mesocosm case: 1–1 treatment in 60 L size in Germany (Ref. 20), 2–2 treatments in 50 L size in USA (Ref. 16), 3–1 treatment in 12 L size in Canada (Ref. 19), 4–1 treatment in 60 L size in Germany (Ref. 25), 5–6 treatments in 700 L size in USA (Ref. 18), 6–15 treatments in 1.1 ML size in USA (Ref. 17), 7–1 treatment in 950 L size in Switzerland (Ref. 22), 8–3 treatments 950 L size in Switzerland (Ref. 22), 9–This study (drift).
tion scenarios (i.e., frequency, amount, composition, etc.). Because synthetic microcosm systems are relatively easier to handle than naturally derived microcosms, they are considered useful for various purposes such as range finding and supplemental for the verification laboratory standard tests as well as more complex higher-tier studies (outdoor microcosms/mesocosms).

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