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TOLERANCE OF SEWAGE TREATMENT PLANT MICROORGANISMS TO MOSQUITOCIDES

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ABSTRACT. Beneficial protozoa and rotifers collected from a wastewater treatment plant in Panama City, FL, were tested for tolerance to 11 commonly used mosquito larvicides and adulticides in the laboratory. The acute effects were assessed using selected concentrations of the adulticides fenthion, malathion, naled, permethrin, and resmethrin; and the larvicides *Bacillus thuringiensis israelensis, Bacillus sphaericus,* diflubenzuron, larviciding oil, methoprene, and temephos for the following microorganism taxa: ameoboids, flagellates, free-swimming ciliates, stalked ciliates, and rotifers.

Protozoa and rotifers are vital components of wastewater treatment processes. These organisms consume bacteria in secondary effluents and flocculate suspended matter (Gerardi 1986). In addition, some protozoa degrade organic waste and enhance the nitrification process (Bolton and Klein 1976).

Microbial survival may be jeopardized by contaminants drained into domestic wastewater as well as factors within treatment facilities (Tchobanoglous and Burton 1991). Contaminants include organic compounds such as pesticides used by agriculture and industry. Other factors that can affect the microbes are internal to the facility, such as elevated pH due to high ammonia concentrations, low temperatures, dilution effects due to rainfall, etc. Managers of treatment facilities utilizing biological reduction systems must regulate contributors of xenobiotics that may adversely affect their operation.

"General Pretreatment Regulations for Existing and New Sources of Pollution" (EPA 40 CFR 403 [1992]) require "Industrial Users" to monitor their effluents to "Publicly Owned Treatment Works" (POTW). Nonindustrial contributors such as entomological research facilities that dispose of substances classified as "prohibitive discharge" with domestic wastewater may be requested to study their toxic effluents. The purpose of this study was to determine effluent limits of selected mosquitocides in order to satisfy permitting requirements. Methods developed by Briton and Greason (1989) were not utilized, instead original in-house methods were developed.

This paper reports the tolerance of beneficial protozoa and rotifers to 11 commonly used mosquito control compounds (mosquitocides) at worst-case scenario concentrations, that is, maximum concentrations expected to be used in laboratory bioassays.

Treatment plant microbes were sampled from an aerated primary liquor by an operator at the St. Andrew Wastewater Treatment Plant in Panama City, FL. The 2-liter sample was transported to the John A. Mulrennan, Sr. Research Laboratory (JAMSRL), where it was immediately aerated and enclosed in an acrylic box to keep the aerosol from contaminating the area. A single 2-liter sample provided sufficient material for up to 5, 24-h tests.

The following mosquitocides were used in bioassays against the microbes: (adulticides) fenthion, malathion, naled, permethrin, and resmethrin and (larvicides) *Bacillus thuringiensis israelensis, Bacillus sphaericus,* diflubenzuron, larviciding oil (GB-1111), methoprene, and temephos. To obtain the appropriate concentration, adulticides were serially diluted in acetone and larvicides were diluted in deionized water, except for GB-1111, which was not diluted. One milliliter of test solution was added to each beaker to obtain the desired concentration. The effect of acetone (1 ml in 500 ml water) was also tested due to its use as a diluent for most of the above compounds. Test concentrations were based on 10 and 100 times the calculated maximum concentration in 15.24 cm (6 in.) of water, rates commonly used as the upper limit for in-house bioassays at JAMSRL. Each test was accompanied by a control for comparison.

Bioassays were run in 500 ml beakers immersed in a water bath (20°C) for 24 h. Prior to treatment, microbes suspended in 50 ml of primary liquor were added to 450 ml of well water in each beaker. Each treatment was replicated 3 times. Due to procedural error, the 3 replicates for larvicide tests were combined before sampling for microbial mortality; adulticide test samples were assessed individually. If there was obvious microbial mortality, additional tests were conducted using lower concentrations. Microbial survival was assessed by transferring a drop of sample from the settled organic layer in the test

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Table 1. Beneficial microbe taxa[1] identified in primary liquor samples from the St. Andrew Wastewater Treatment Plant, Panama City, FL.

Phylum Protozoa
- Class Ciliata
  - Subclass Holotrichia (free-swimming ciliates)
    - *Lionotus fasciola*[2]
    - *Trachelophyllum* sp.[2]
  - Subclass Spirotrichia
    - *Stentor polymorphus*
    - *Spirostomum teres*[2]
    - *Halteria grandinella*
    - *Euplotes patella*[2]
  - Subclass Suctoria
    - *Acineta* sp.
  - Subclass Peritrichia (stalked ciliates)
    - *Vorticella* sp.
    - *Opercularia* sp.
    - *Carchesium* sp.
- Class Mastigophora (flagellates)
  - *Paranema trichophorum*[2]
  - *Euglena* sp.
  - *Volvox globator*
- Class Rhizopoda (amoeboids)
  - Subclass Sarcodina
    - *Amoeba proteus*[2]
    - *Arcella vulgaris*
    - *Diflugia* spp.
    - *Centropyxis aculeata*[2]

Phylum Rotataria (rotifers)
- *Rotaria* sp.
- *Cephalodella* sp.
- *Enteroplea lacustris*

[1] Based on Gerardi (1986) if not mentioned in footnote 2, then identified at JAMSRL.
[2] Identified at wastewater treatment plant.

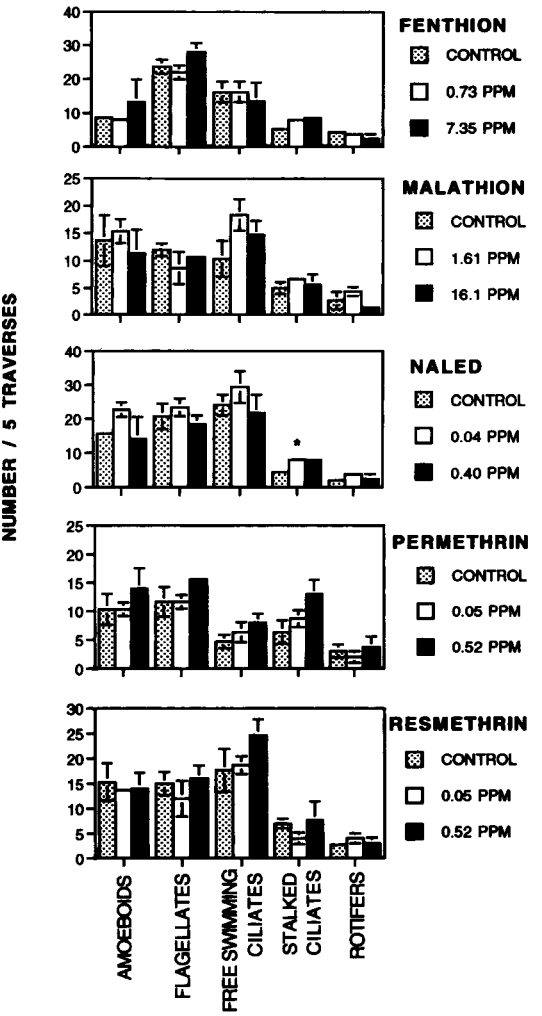

Fig. 1. Average abundance (±SE) of 5 taxa of microorganisms after 24-h exposure to mosquito adulticides. Asterisk indicates significant difference ($P < 0.05$) in abundance within the taxon.

beaker to a glass slide and observed under a compound microscope (Nikon Biophot) at a magnification of 100×. Numbers of live or motile ameoboids, flagellates, free-swimming ciliates, stalked ciliates, and rotifers were enumerated. Comparisons were based on either total number of organisms in each group occurring in the drop (larvicide tests) or number in 5 traverses across the slide (adulticide tests). Effects of adulticides were analyzed using *t*-tests in a general linear model (SAS Institute 1985).

A total of 20 microbial genera were found in the primary liquor, either identified at JAMSRL or at the St. Andrew Wastewater Treatment Plant (Table 1).

Exposure to each larvicide at initial concentrations (Table 2) had no noticable effect on the survival of amoeboids, flagellates, free-swimming ciliates, stalked ciliates, or rotifers. Of the adulticides, naled was the only compound where 100% mortality was observed at the initial concentration tested (i.e., 4.0 ppm). It was further tested at a concentration reduced by one order of magnitude and no significant mortality was detected (Table 2 and Fig. 1). As indicated by the asterisk, stalked ciliates were significantly more abundant in naled treatments compared to controls (Fig. 1).

Ruber and Jobbins (1961) studied the effect of naled (1.4 and 1.75 lb/acre) on *Euglena* sp. and Rotifera in 1-acre field plots. Rotifera were recovered more frequently in treatment plots than

Table 2. Concentrations of mosquito larvicides and adulticides having no acute toxicity to wastewater microorganisms.

Compound/microbial	Maximum concentration[1] (ppm)
Larvicides	
Bacillus thuringiensis israelensis	237.4
Bacillus sphaericus	78.5
Diflubenzuron	3.7
Larviciding oil (GB-1111)	2,625.0
Methoprene	1.0
Temephos	3.4
Adulticides	
Fenthion	7.3
Malathion	16.1
Naled	0.40
Permethrin	0.52
Resmethrin	0.52
Solvent	
Acetone	2,000.0

[1] Based on 100× expected concentration in 15.2 cm (6 in.) of water.

in controls, whereas protozoa were rare in both cases. In another study, Moore (1970) found that growth and survival of the protozoan *Euglena gracilis* was reduced 49% by malathion at a concentration of 7.25 ppm based on coulter counter assessments. This concentration is well below the 16 ppm determined to be nontoxic in the current study. The disparity may be due to a longer exposure interval (5 days) or to individual differences in colony susceptibility. A third reason may be that our test water was higher in organic content. Compounds are often adsorbed onto organic debris in the water as reported for diflubenzuron (Hester et al. 1986).

Rotifer susceptibility has been studied using methoprene (Schaefer et al. 1974), permethrin (Day 1989), diflubenzuron (Miura and Takahashi 1975, Apperson et al. 1978), and malathion (Fernandez-Casalderry et al. 1992). The latter authors determined a 24-h LC_{50} of 33.72 ppm for the rotifer *Brachionus calyciflorus* exposed to malathion. We found no apparent rotifer mortality at 16.1 ppm. The remaining authors, however, studied much lower concentrations typical for aquatic mosquito control and reported no deleterious effects to rotifers.

In conclusion, dispensing the above mosquitocidal compounds into domestic wastewater at the tabulated concentrations would not be expected to adversely affect the beneficial microbes at the wastewater treatment facility. The possibility of exposing sewage treatment plant microbes to our "worst-case" bioassay concentrations is highly unlikely due to dilution effects.

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