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APPARENT DRUG RESISTANCE TO THE ORGANOPHOSPHATE DIMETHYL (2,2,2-TRICHLORO - 1 - HYDROXYETHYL) PHOSPHONATE BY MONOGENETIC TREMATODES

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Abstract: Gyrodactylus elegans on goldfish, *(Carassius auratus)* from a commercial farm were resistant to recommended dosages of dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate. Controlled experiments suggest that a dosage 100 times the commonly recommended minimal dosage (.25 mg/l) was required to remove trematodes. A hypothesis is proposed to account for the development of drug resistant trematodes based on the life cycle of the parasites and continual drug exposure.

INTRODUCTION

One of the most commonly used treatments to control anchorworms *(Lernea sp.),* fish lice *(Argulus sp.)* and monogenetic trematodes *(Gyrodactylus* sp., *Dactylogyrus* sp., and *Cleidodiscus* sp.) is dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate, sold under trade names of Dylox,[3] Masoten,[4] and Combot.[5] The effective control of *Gyrodactylus* sp. in golden shiners *(Notemigonus crysoleucas),* and *Dactylogyrus* sp. in fathead minnows *(Pimephales promelas)* required 0.25 mg/l of active trichlorfon in aquaria.[6] Higher concentrations of 0.4 mg/l and 0.8 mg/l have been used to remove *Dactylogyrus* sp. from carp *(Cyprinus carpio).*[7,8]

Observations in our laboratory, of various lots of goldfish *(Carassius auratus)* from ponds where organophosphates had been used for years, suggested that the above dosages did not remove *Gyrodactylus elegans* under controlled experimental conditions. The objective of this study was to determine the degree of resistance by measuring the amount of trichlorfon required to remove *Gyrodactylus* from pond-reared goldfish.

METHODS

Common goldfish procured from a commercial hatchery, were untreated prior to shipment and examined for trematodes on arrival. "Young of the year" channel catfish, *Ictalurus punctatus,* were obtained from a commercial farm where Masoten is not in use.

The trematodes *(Cleidodiscus* sp.) infesting these fish represented a parasite population that was not continually exposed to the organophosphate. All fish were maintained in 16 l glass aquaria equipped with undergravel filters. Aeration was provided from a central compressed air supply attached to the air lift system of the gravel filters.

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[3] Dylox^{R} Chem Agro Corp. Division of Baychem Corp., Box 4913, Kansas City, Missouri 64120, USA.

[4] Masoten^{R} Chem Agro Corp. Division of Baychem Corp., Box 4913, Kansas City, Missouri 64120, USA.

[5] Combot^{R} Haver-Lockhart Laboratories, Box 390, Shawnee, Kansas 66201, USA.

A liquid formulation of dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate was used as the treatment. Analysis of the drug by gas chromatography showed 6.25% active trichlorofon.[5] Fish were exposed to long term baths of various concentrations of drug. Dosages ranged from 0.2 mg/l to 100 mg/l of active drug. Fish were treated 72 h and then examined for trematodes.

Water quality parameters measured were total hardness, 72 mg/l (Ca 62 mg/l; Mg 10 mg/l) and pH 7.4. Water temperatures ranged between 18-20 C. Ammonia and nitrite levels checked periodically during the experiment, were below 1 mg/l and 0.1 mg/l respectively.

RESULTS

The recommended dosage of 0.25 mg/l was not effective in removing *Gyrodactylus elegans* from goldfish under the experimental conditions (Table 1). Increasing concentrations were not effective until dosages of 25 mg/l were used. This concentration removed all trematodes in 66.6% fish in Trial I, and was 100% effective in Trial II. A 50 mg/l dosage removed all trematodes from 13 of 14 fish in Trial I and was 100% effective in Trial II. No mortality occurred at these dosages. In both trials, fish held at a concentration of 100 mg/l died within 24 h of treatment.

None of the catfish treated with the recommended dose of 0.25 mg/l had trematodes when compared to untreated catfish controls. This result indicates that this population of trematodes was not resistant to the action of the drug.

DISCUSSION

Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate has been extensively used for the past 13 years as a treatment for monogenetic trematodes.[2] Our observations suggest that the recommended dosage of the drug is no longer effective in controlling *Gyrodactylus* sp. on fish from ponds treated with the drug over a period of years. Dosages as high as 100 times greater than the initial recommended dosages are now required to remove the trematodes. One possible reason is that the trematodes have become resistant to the action of the drug. In experimental conditions, the increased dosage (25 mg/l) approaches the toxic level of the drug (100 mg/l). Other studies have shown the toxic dose to be considerably lower; for example, Meyer reported that the 48 hour LC_{50} for goldfish was 45 mg/l.[5]

Reduced susceptibility to the toxicant can be discussed in terms of tolerance and resistance. The basic mechanism of tolerance to this drug is unknown. However, the induction of tolerance appears to be associated with previous or continuing exposure to low concentrations. One of the major *in vivo* degradation products of trichlorfon is the biologically ineffective but persistent desmethyltrichlorfon. The persistence of this metabolite may act as a stimulus for increasing enzyme concentrations, which could result in the organisms' increased ability to metabolize the parent compound.[4] This type of tolerance frequently is observed in pesticides such as dieldrin.[3] It has been clearly shown that the increased metabolism responsible for this tolerance is mediated by increased activity of the microsomal mixed function oxidases, which are induced by pesticides.[3] As indicated by the results of this experiment, fish not previously exposed to the drug did not harbor resistant populations of trematodes.

Resistance refers to relative insusceptibility that is genetically determined. The genetic trait may preexist in the population or may be brought to an

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TABLE 1. Number of fish infested with *Gyrodactylus elegans*/total number of fish examined, 72 h post treatment with Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate.

	Number of Fish Infested	
Dosage mg/l	Trial I % Infestations	Trial II % Infestations
0.2	10/10 (100%)	
0.4		19/19 (100%)
0.8	11/11 (100%)	20/20 (100%)
1.3		19/19 (100%)
3.0	10/10 (100%)	20/20 (100%)
6.0		13/20 (65%)
12.5	11/11 (100%)	8/21 (38%)
25.0	4/12 (33%)	0/6 (0%)
50.0	1/14 (7%)	0/6 (0%)
Control	11/13 (85%)	21/21 (100%)

observable level through selection. Resistance may be due to a variety of factors including difference in metabolism, membrance permeability or enzyme induction. Resistance to a particular compound may not involve an entire species but only a population of a limited area.[3]

A hypothesis proposed for the mechanism of development of resistance relates to the life cycle of *Gyrodactylus* sp. In the development of this viviparous trematode, an embryo forms within the parent, and within that embryo appears another, or third generation. On completion of development, the first embryo passes to the outside, and develops into an adult, then the second embryo within the first begins maturation.[1] Through this system of polyembryony, immature trematodes may be continually exposed to low concentrations of the drug while *in utero*, from constant exposure to sublethal concentrations of drug. Alternatively, genetically determined resistance may develop by mutation during the course of exposure, allowing the organisms to develop a genotype that can cope with the toxicant.

This work suggests that drug resistance of monogenetic trematodes is a potential problem in aquaculture. Based on our data, it appears that new drug formulations are indicated to supplement present drugs, as a means of minimizing this developing problem.

A practical treatment regime, designed to circumvent the development of drug resistant parasites, could be to use combinations of parasiticides or to use alternating treatments of drugs with different mechanisms of action.

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