Chronic Toxicity of Guthion to the Fathead Minnow (*Pimephales promelas* Rafinesque)

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INTRODUCTION

Guthion (azinphosmethyl) is widely used as an insecticide on deciduous fruits and cotton as well as on many other fruits and vegetables. Although acute toxicity data have been reported by many workers for a variety of fish species (KATZ 1961, PICKERING et al. 1962, MEYER 1965, and MACEK and MCALLISTER 1970), no information has been reported on the effect of exposures over a complete life cycle. Since data on chronic toxicity are important for establishment of water quality standards, fathead minnows were exposed to various concentrations of Guthion over a complete life cycle, from egg through fry in the second generation, in order to determine a maximum acceptable toxicant concentration (MATC) (MOUNT and STEPHAN 1967).

MATERIALS AND METHODS

Three experiments (I, II, and III) were conducted. Tests I and II were terminated after 26 and 68 days, respectively, when it became apparent that the lowest concentration was causing significantly greater mortality than the control and test III was conducted for 250 days.

Toxicant was administered by a proportional diluter (MOUNT and BRUNGS 1967) which dispensed seven toxicant concentrations and one control. Technical grade Guthion was added directly to a single mixing chamber by the injector described by DE FOE (1975). An air dispersion stone and baffles thoroughly mixed the Guthion with incoming water before it was siphoned to the toxicant cells in the diluter. The test chambers for first generation young and adult fish were glass (50 x 25 x 20 cm) and contained 20 liters. A flow rate of 250 ml/min replaced 90% of the water in approximately 3-1/4 hr. A temperature of 25 C was maintained by a hot water exchanger and water was aerated in the head tank. All test chambers in test III contained an air dispersion stone. A dense bacterial growth occurred in the two highest concentrations, apparently using the Guthion as a nutrient, and aeration was required to replace oxygen consumed by the bacteria. Chambers were siphoned daily except when fry were present. A complete analysis of hard water from the deep laboratory well (210 mg/liter $CaCO_3$ total hardness) was reported by ADELMAN and SMITH (1972) and test conditions in all chambers are listed in Table 1.

Fry in the second generation were reared in glass chambers

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TABLE 1

Mean and range of routine water quality analyses

Test	Temperature (C)	pH meter reading	Dissolved oxygen (mg/liter)
I - mean	25.0	7.54	5.30
range	23.9-26.6	7.38-7.91	3.15-7.40
II - mean	24.9	7.61	5.45
range	17.0-25.8	7.30-7.95	4.50-7.35
III			
adult			
mean	24.4	8.06	6.20
range	19.0-25.4	7.68-8.24	3.75-7.50
fry chamber 1			
mean	23.7	8.16	6.52
range	21.5-24.6	8.03-8.31	4.30-7.85
fry chamber 2			
mean	22.9	8.17	6.79
range	18.0-24.7	8.03-8.32	5.25-7.90

(20 x 20 x 21) containing 6.4 liters. For each concentration water flowed from the outlet of the adult test chamber into the first fry chamber, then from the outlet of that to a second fry chamber so that two groups of offspring from the same adults could be reared simultaneously. Eggs were hatched in a jar with a nylon screen bottom. One jar in each adult test chamber was oscillated so that water flowed past the eggs without raising them from the screen.

Fathead minnows obtained from the National Water Quality Laboratory at Duluth, Minnesota were reared in the laboratory. All tests were started with pooled eggs from five spawning pairs which were randomly distributed to egg chambers in each toxicant concentration and control. Initial numbers of eggs were 90, 70, and 100 in tests I, II, and III, respectively. Eggs were in the middle to late blastula stage when first exposed to the toxicant and more than 99% were fertile. Mortality of eggs and fry was recorded at 24-hr intervals in tests I and II. In test III eggs were checked at 24-hr intervals but survival of young was recorded after 22 days to avoid stress from handling. At that time, two runts or malformed fish were removed from each chamber and fish were randomly thinned to 30 per chamber. After 60 days each chamber was again randomly thinned to 15 fish.

Ten-cm sections of transite pipe were split lengthwise to form a spawning substrate. After 90 days six sections were placed in each aquarium, three on each end with an open area in the center. As secondary sexual characteristics developed, males were removed biweekly to keep no more than five per chamber.

Tiles were checked at 1000 hours each day and if spawning occurred, eggs were counted and a random sample of 50 was placed in the egg basket for determination of survival unless the basket was already occupied. Two groups of fry from each concentration were reared for 50 days.

Fry were fed ground Glencoe trout pellets and live brine shrimp twice a day for the first 30 days. Older fish were fed Oregon Moist frozen trout pellets twice a day and live brine shrimp once a day.

Guthion was analyzed by gas chromatograph after extraction into benzene, and results were corrected for the recovery efficiency and technical grade product. Concentrations were usually analyzed once or twice per week. Since no measurable loss of Guthion occurred between the adult chambers and either fry chamber, analyses from the former were used. The pH was determined twice per week in adult chambers and once per week in fry chambers when they were occupied. Temperature was measured three times per week in all chambers and dissolved oxygen was analyzed weekly by the azide modification of the Winkler analysis.

RESULTS

Egg and Fry Survival

First Generation--In test I percentage survival through hatching decreased from 90 to 29 to 17% as Guthion concentration increased from 34.5 to 61.4 to 96.9 µg/liter but survival increased to 76% as concentration increased to 165.9 µg/liter (Table 2). The cause of this decreased survival was due to eggs in these concentrations being enmeshed in a bacterial network that apparently used Guthion as an energy source and grew in proportion to the concentration. Survival was reduced because hatching fry became enmeshed in the bacteria and could not break free. Since the chorion of these eggs softened, fry may also have been weakened. Survival in the highest concentration was apparently greater because most eggs hatched 1 day sooner, when the bacteria were less dense and before the chorion had softened.

At 22 days survival of fry decreased in comparison to controls at 7.6 µg/liter in test I, and at 6.5 µg/liter in test II (Table 2). By 57 days survival decreased at 1.8 µg/liter Guthion in test II but did not decrease at 0.51 µg/liter in test III. In test III Guthion caused no decrease in survival at any life history stage, with the possible exception of the highest concentration where fry were lost when the chamber overflowed. The MATC in terms of survival occurred between 0.51 µg/liter in test III and a maximum of 1.8 µg/liter where increased mortality occurred in test II.

Growth

In test II fish were weighed twice en masse during the experiment and at termination. Each time the control fish weighed more than those in 1.8 µg/liter, the lowest concentration (Table 3).

TABLE 2

Percentage survival of fathead minnow eggs and young exposed to various concentrations of Guthion (standard deviation in parentheses)

				Test I				
Guthion (µg/l)	0	7.6 (1.3)	11.7 (1.9)	20.5 (1.9)	34.5 (3.2)	61.4 (2.4)	96.9 (6.6)	165.9 (13.1)
% hatched	99	84	97	95	90	29	17	76
% survival								
22 days	54	43	29	16	1	0	0	0
				Test II[a]				
Guthion (µg/l)	0	1.8 (0.9)	1.9 (1.0)	2.8 (1.1)	3.4 (1.2)	4.8 (0.8)	6.5 (0.8)	15.0 (2.2)
% hatched	91	92	90	90	87	93	63	84
% survival								
22 days	57	48	57	43	48	48	37	35
57 days	54	42	34	5	3	0	0	0
98 days	52	35	21	0	0	0	0	0
				Test III				
Guthion (µg/l)	.04[b] (.03)	.10 (.04)	.16 (.05)	.24 (.06)	.33 (.08)	.51 (.11)	.72 (.15)	- -
% hatched	86	84	82	85	85	86	83	-
% survival								
22 days	61	80	74	82	76	67	[c]	-
from 22-60 days	100	100	93	100	100	93	88	-

[a] Concentrations may be high by as much as 50% for the four lowest concentrations.
[b] Unknown source of contamination resulted in some Guthion in the control.
[c] Tank overflowed and fry were lost.

In test III individual t-test comparisons indicated no significant difference between the control and any of the treatments ($p > .05$) at 22 days (Table 3). At 60 days, the first and third treatment levels weighed significantly more than the controls ($p < .02$) but since the second treatment level was not different than the control, Guthion was probably not the cause of these differences. After 90 days there was no significant difference ($p > .05$) between the control and any treatment.

By 120 days males in some treatments started to defend territories and fish were not weighed to avoid any influence of handling on spawning behavior. When the experiment was terminated no consistent pattern of growth related to Guthion concentration was

TABLE 3

Mean weight of fathead minnows at various time intervals after spawning (number of fish weighed in parentheses)

			Test II					
Guthion (µg/l)	0	1.8	1.9	2.8	3.4	4.8	6.5	15.0
Weight (mg)								
57 days	162 (33)	111 (29)	55 (21)	72 (3)	20 (2)	-	-	-
85 days	320 (33)	250 (24)	230 (14)	-	-	-	-	-
98 days	380 (33)	350 (24)	300 (13)	-	-	-	-	-
			Test III					
Guthion (µg/l)	.04	.10	.16	.24	.33	.51	.72	
Weight (mg)								
22 days [a]	24 (8)	24 (27)	28 (25)	27 (19)	23 (21)	21 (11)	-	
60 days [a]	179 (15)	242 (15)	216 (13)	231 (15)	260 (15)	165 (13)	-	
90 days [b]	580 (15)	580 (15)	640 (15)	660 (14)	600 (15)	600 (13)	720 (15)	
250 days [c]								
male	2834 (3)	3047 (5)	2455 (5)	3051 (5)	2628 (5)	2965 (6)	3136 (5)	
female	1434 (9)	1594 (7)	1458 (5)	1353 (5)	1558 (5)	1360 (6)	912 (1)	

[a] Fish randomly thinned from experiment.
[b] Fish remaining in experiment.
[c] Termination.

observed. Therefore, Guthion affected growth of fatheads through 90 to 100 days at levels between 0.72 and 1.8 µg/liter.

Reproduction

Fecundity--Interpretation of fecundity data was complicated by the variation in sex ratios in test chambers. Although an attempt was made to maintain five males and seven females in each chamber, in some chambers only a few males were produced, in some immature males could not be identified, and in some individuals were lost to accidental death or escape.

Intensive spawning occurred first at 0.33 µg/liter Guthion, followed 12 to 14 days later at 0.24, 0.16, and 0.10 µg/liter. Intensive spawning by the control fish occurred 29 days later and

56 days later in 0.51 and 0.72 µg/liter Guthion although at 0.51 µg/liter spawning occurred infrequently during the test.

The numbers of spawnings and eggs were greatest in the control chambers (Table 4). The more significant measurements in terms of Guthion effect were the number of eggs per spawning and per female. On this basis Guthion affected fecundity between 0.33 µg/liter which was similar to the controls and 0.51 µg/liter which produced fewer eggs per spawning and per female (Table 4).

TABLE 4

Fecundity of fathead minnows exposed to various concentrations of Guthion

Guthion (µg/l)	Number of spawnings	Number of eggs	Number of eggs per spawning	Number of eggs per female[a/]
.04	88	16401	186	1691
.10	54	8541	158	1220
.16	57	9567	168	1611
.24	49	7091	145	1239
.33	52	8590	165	1718
.51	21	1791	85	256
.72	27	2408	89	782

[a/] Computed by intervals between thinnings.

Second Generation

Random samples of 50 eggs were taken from 12 spawnings from each test chamber except the second highest, where seven were taken. These eggs were hatched in the oscillating egg baskets with no effect of Guthion at the test concentrations(Table 5).

Survival or growth of fry was not consistently affected by tested Guthion concentrations (Table 5). Survival was poorest in controls and no cause for this can be offered. Considerable differences in growth occurred between test chambers but these differences were not related to Guthion concentrations.

DISCUSSION

The lowest concentration of Guthion that had an adverse effect was 0.51 µg/liter where fecundity was drastically reduced (Table 4). An estimation of the maximum acceptable toxicant concentration based on this parameter would be between 0.33 and 0.51 µg/liter Guthion. Effects on survival and growth during early life history stages occurred at slightly higher concentrations (Tables 2 and 3). Although no effect occurred in test III, growth and survival were reduced at the lowest concentration in test II. Combining the two tests sets the MATC between 0.51 and 1.8 µg/liter Guthion, although the latter figure may be high by as much as 50%.

TABLE 5

Effect of Guthion on survival and growth of second generation eggs and fry

Item	Guthion concentration (µg/liter)						
	.04	.10	.16	.24	.33	.51	.72
% survival							
eggs	86.2	84.2	90.9	86.2	82.3	86.4	90.2
fry							
1st group	79	97	100	97	89	[a]	78
2nd group	69	89	88	89	100	-	100
Weight (mg) at 50 days							
1st group	94	130	94	126	115	78	128
2nd group	143	102	135	85	144	222	80

[a] Some fry lost due to overflow.

The 96-hr LC50 for 11-week-old fathead minnows tested in a soft water was 1900 µg/liter (ADELMAN et al. 1976). An application factor based on this acutely toxic concentration and the MATC in the present study would be between .00017 and .00027. This application factor is extremely small compared to factors found for many other toxicants (U.S. ENVIRONMENTAL PROTECTION AGENCY 1972). HENDERSON et al. (1960) found that the 96-hr LC50 of Guthion to fathead minnows was approximately 25 to 40% less in soft water than in hard (20 mg/liter vs. 400 mg/liter total hardness). This differential in hardness was more extreme than the acute vs. chronic test in the present study but if the application factor was based on an acute test conducted in hard water, the implication is that the factor would be even smaller.

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REFERENCES

ADELMAN, I.R., and L.L. SMITH, JR.: J. Fish. Res. Board Can. 29, 1309 (1972).
ADELMAN, I.R., L.L. SMITH, JR., and G.D. SIESENNOP: J. Fish. Res. Board Can. in press (1976).
DE FOE, D.L.: J. Fish. Res. Board Can. 32, 544 (1975).
HENDERSON, C., Q.H. PICKERING, and C.M. TARZWELL: In Biological Problems in Water Pollution. Trans. 1959 Seminar. R.A. Taft Sanit. Eng. Center, Cincinnati, Ohio (1960).
KATZ, M.: Trans. Amer. Fish. Soc. 90, 264 (1961).
MACEK, K.J., and W.A. MCALLISTER: Trans. Amer. Fish. Soc. 99, 20 (1970).
MEYER, F.P.: Trans. Amer. Fish. Soc. 94, 203 (1965).
MOUNT, D.I., and W.A. BRUNGS: Water Res. 1, 21 (1967).
MOUNT, D.I., and C.E. STEPHAN: Trans. Amer. Fish. Soc. 96, 185 (1967).
PICKERING, Q.H., C. HENDERSON, and A.F. LEMKE: Trans. Amer. Fish. Soc. 91, 175 (1962).
U.S. ENVIRONMENTAL PROTECTION AGENCY: Ecol. Res. Ser., EPA-R3-73-033 (1973).