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The toxic effects of captan on survival, growth, and reproduction of fathead minnows (*Pimephales promelas*) and on survival of bluegills (*Lepomis macrochirus*) and brook trout (*Salvelinus fontinalis*) were determined in a flow-through system. In a 45-week exposure of fathead minnows, survival and growth were adversely affected at 39.5 μg/liter. Adverse effects on spawning were suspected but not statistically demonstrated at 39.5 and 16.5 μg/liter. The maximum acceptable toxicant concentration (MATC), based on survival and growth, lies between 39.5 and 16.5 μg/liter. The lethal threshold concentration (LTC) derived from acute exposures was 64 μg/liter, resulting in an application factor (MATC/LTC) between 0.26 and 0.62. LTC values for the bluegill and brook trout were 72 and 29 μg/liter, respectively. The estimated MATC is between 44.6 and 18.7 μg/liter for the bluegill and between 18.0 and 7.5 μg/liter for the brook trout.

The half-life of captan in Lake Superior water with a *p*H of 7.6 is about 7 hr at 12 C and about 1 hr at 25 C. Breakdown products from an initial 550 μg/liter of captan were not lethal to 3-month-old fathead minnows.

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Nous avons mesuré les effets toxiques du captan sur la survie, la croissance et la reproduction du tête-de-boule (*Pimephales promelas*) et sur la survie du crapet arlequin (*Lepomis macrochirus*) et de l'omble de fontaine (*Salvelinus fontinalis*) dans un système à flot continu. Une exposition de 45 semaines à une concentration de 39.5 μg/litre affecte la survie, la croissance et la reproduction du tête-de-boule. Une concentration de 16.5 μg/litre réduit la reproduction. La concentration maximale acceptable du toxique (MATC), basée sur le nombre de fraies et le nombre d'oeufs par femelle, se situe entre 39.5 et 16.5 μg/liter. La concentration de seuil létale (LTC), déterminée par expositions aiguës, est de 64 μg/litre, ce qui donne un indice d'application (MATC/LTC) de 0.26 à 0.62. Les valeurs de LTC pour le crapet arlequin et l'omble de fontaine sont de 72 et 29 μg/litre respectivement. La MATC estimée se situe entre 44.6 et 18.7 μg/litre pour le crapet arlequin et 18.0 et 7.5 μg/litre pour l'omble de fontaine.

La demi-vie du captan dans l'eau du lac Supérieur, d'un *p*H de 7.6, est d'environ 7 h à 12 C et 1 h à 25 C. Les produits de dégradation d'une concentration initiale de 550 μg/litre de captan ne sont pas mortels pour des têtes-de-boule âgés de 3 mois.

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CAPTAN (*cis*-N (trichloromethyl)thio-r-cyclohexene-1,2-dicarboximide)), one of the phthalimide fungicides, is used widely throughout the world. Little information is available, however, concerning the effects of this fungicide on aquatic life. Acute studies by Abedi and McKinley (1967) have demonstrated that 1.0 mg/liter of captan will kill larval zebrafish (*Brachydanio rerio*) in 90 min. Holland et al. (1960) found that 0.32 mg/liter of Captan 50-W (50% captan–50% inert material) killed 50% of exposed rainbow trout (average size 4.8 inches) within 72 hr. A 26-hr LC50 value of 1.30 ppm for *Daphnia magna* was observed by Frear and Boyd (1967). The above experiments were carried out in static water systems. No in-

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formation on flow-through exposures of aquatic organisms to captan could be found in the literature. The purpose of this study was to determine, in a flow-through system, the toxicity of captan to fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*), and brook trout (*Salvelinus fontinalis*). Chronic and acute toxicities were determined for fathead minnows, and acute toxicity was determined for bluegills and brook trout. An application factor (Mount and Stephan 1967; Eaton 1970) derived from the fathead minnow chronic and acute data was used to estimate the chronic toxicity values for bluegills and brook trout.

Materials and Methods

Chronic Exposure

The design of the chronic exposure study closely followed procedures recommended by the National Water Quality Laboratory Committee on Aquatic Bioassays (Bioassay Committee 1971).

Physical — A proportional diluter and syringe injector (Mount and Brungs 1967) delivered test water and toxicant to the test chambers. A control and five captan concentrations flowed to their respective 30.5- × 30.5- × 61-cm duplicate spawning chambers and 44- × 15- × 18-cm quadruplicate larval chambers. The volumes of water in the spawning and larval chambers were 28.3 and 8.7 liters, respectively. All chambers were separated from one another, and each received a flow of 10.5 tank volumes per 24 hr. The flow rate was selected to maintain the dissolved oxygen above 65% saturation in all chambers.

Before entering the diluter, Lake Superior water was filtered, sterilized with ultraviolet light, and warmed to about 25 C in a stainless steel headbox by a coiled stainless steel heat exchanger. A solenoid valve activated by a thermoregulator relay system (Syrett and Dawson 1972) controlled the flow of hot city water through the heat exchanger. Temperatures in all test chambers were checked daily, and one chamber was monitored continuously with a recording thermometer.

The photoperiod was adjusted the 1st and 15th day of each month, following the normal daylight hours of Evansville, Indiana (represents average U.S. day lentgh). The daylight length at the start of the experiment coincided with the first week of December in Evansville. Because the fish developed more slowly than expected, the peak photoperiod of 15 hr and 15 min was extended and additional 5 weeks to assure them of a 4-month spawning period. Light intensity at the water's surface ranged from 27 to 36 ft-c for the spawning chambers and 33–46 ft-c for the larval chambers.

Chemical — A stock solution containing, by weight, 1.94% technical grade captan (88.4% purity), 0.05% of the surfactant Triton X-100[2], and 98.01% acetone was delivered to the dilution water from a 50-ml glass syringe and Teflon® needle. The outlet of the needle was submersed in the chamber water and attached to an air stone to promote dissolution of the captan. No amounts of acetone or Triton X-100 were added to the control water while the highest amounts were added to the high concentration (63.5 μg/liter), and they never exceeded 14.9 mg/liter and 6.7 μg/liter, respectively. Brungs (personal communication) found that 870 mg liter of acetone in an 80-day exposure and 1.0 mg/liter of Triton X-100 in a 30-day exposure have no deleterious effects on the fathead minnow. In spite of the acetone, Triton X-100, and air agitation, about 12% of the captan (determined by gas-chromatographic analyses) precipitated and collected at the outlet of the Teflon® needle. The precipitate was discarded each day. The original nominal concentrations of captan were increased twice during the experiment. After 3 weeks they were increased by 100% because no difference in survival or growth of the young fish was apparent, and after the 17th week they were increased by 25% because of a measured decrease in all concentrations starting on the 4th week. The captan concentrations between 3 and 45 weeks (termination of experiment) were:

Nominal concn (μg/liter)	Measured concentration (μg/liter) No. of samples	Mean	Standard deviation	Range
200–250	36	63.5	25.4	16.2–118.2
100–125	63	39.5	12.4	15.9–68.2
50–62.5	63	16.8	4.93	6.9–27.6
25–31.3	63	7.4	2.22	2.9–12.9
12.5–15.6	63	3.3	1.12	1.1–6.8

Captan concentrations were measured once each week for the first 11 weeks and twice each week for the last 34 weeks. Measurements of the high concentration were discontinued between the death of all the first generation fish and the beginning incubation of the control embryos in the high concentration. A duplicate and a spiked sample of control water were analysed with each set of samples. Captan was extracted with 20% methylene chloride/petroleum ether and analyzed by gas chromatography as described by the U.S. Environmental Protection Agency (1971). No interfering chlorinated or organophosphate compounds were found in the control water. Fish food (Oregon Moist) fed to the young and adult fish was examined for pesticide content according to methods described by the U.S. Department of Health, Education, and Welfare (1971). Nothing was found above the detection limits of 10 ng/g for organophosphates and 1.0 ng/g for chlorinated hydrocarbons. Chemical characteristics of the test waters determined each week by methods described by the American Public Health Association (1971) were:

[2]Mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

	Mean	Range
Dissolved oxygen	7.2 mg/liter	5.6–8.4
Acidity	3.3 mg/liter	1.8–5.5
Alkalinity	42.0 mg/liter	37–44
Total hardness	45.1 mg/liter	41–49
Temperature	24.9	23.0–26.0
*p*H (median)	7.5	7.1–7.8

Biological — Twenty-five 9-day-old laboratory-cultured fathead minnows were randomly assigned to each of the duplicate spawning chambers (A and B) on September 14, 1971. The fish were photographed as described by McKim and Benoit (1971) after 51 days of exposure to determine extent of growth. On the 51st day, 15 of the surviving fish in each chamber were impartially selected and returned to the spawning chambers. Mature males in excess of five per chamber were removed weekly to reduce territorial competition and were not included in the survival and growth data. All parental fish were sacrificed after 45 weeks of exposure (1 week after cessation of spawning) and were examined to determine sex, gonadal condition, length, and weight.

Groups of 40 1-day-old F_1 fish were reared in the larval chambers for 30 days to determine extent of growth and survival. The young fish were fed Oregon Moist starter three times a day and cultured zooplankton once each day.

Statistical analysis — For statistical evaluation, means of lengths, weights, spawnings/female, eggs spawned/female, and eggs/spawning were transformed to logarithms, and survival data were transformed to arcsin $\sqrt{\%}$ survival. All transformed data were subjected to an analysis of variance ($P=0.05$) and Dunnett's two-tailed comparison of treatment means to control means ($P=0.05$) (Steel and Torrie 1960). A two-way analysis of variance was applied to the growth data of the parental generation to determine if captan affected the growth of one sex more than the other.

Acute Exposure

Flow-through — The chronic-test diluter system was also used for acute exposures of 3.5-month-old laboratory-cultured fathead minnows and 1.5-year-old hatchery-reared bluegills and brook trout. In each experiment 10 fish were randomly assigned to each of the duplicate test chambers. Flow rates were 19 tank volumes/24 hr for the fathead minnows and 22 tank volumes for the bluegills and brook trout. Dissolved oxygen, *p*H, alkalinity, acidity, and total hardness of the test water were determined at the beginning, middle, and end of the experiments. Temperatures were recorded daily. Captan determinations were made each day for the first 5 days. A 96-hr TL50 and a lethal threshold concentration as defined by Eaton (1970) were derived by using the method of Litchfield and Wilcoxon (1949).

Static — A static exposure of fathead minnows was used to demonstrate the breakdown of captan and to determine the acute toxicity of the breakdown products. Two groups of 10 3-month-old fathead minnows were exposed to an initial concentration of 550 μg/liter of captan. The test chamber, a 30.5- × 30.5- × 61-cm aquarium divided by a stainless steel screen, contained 30 liters of 23 C Lake Superior water with an initial *p*H of 7.5. Both sections of the chamber were gently aerated throughout the 10-day exposure. The first group was placed in one section immediately after the toxicant was mixed in the water; the second was placed in the other section 3 hr later.

Results

Chronic Exposure

All but one of the parental fish died at 63.5 μg/liter of captan in 51 days. Survival in the other concentrations, after 51 days and 45 weeks did not differ significantly from the control (Table 1). At 51 days and 45 weeks of exposure, growth in length was significantly different only at 39.5 μg/liter (Table 1). The lone survivor in 63.5 μg/liter was not considered in the 51-day growth analysis because of a possible bias from one resistant individual. A two-way analysis of variance indicated that the growth of both sexes was equally affected by the toxicant. Mean spawnings/female and mean eggs spawned/female appeared to be adversely affected at 39.5 and 16.8 μg/liter. However, due to variability and especially the absence of spawning in one of the 7.4-μg/liter chambers statistical significant spawning differences could not be shown in any of the concentrations (Table 2).

Embryos from unexposed parents incubated in 63.5 μg/liter did as well as control embryos, but all the larvae died 5–8 days after hatching. One hundred percent mortality occurred within 24 hr when 1-day-old larvae from unexposed parents were placed in this concentration. Growth and survival of the F_1 generation were reduced in 39.5 μg/liter after 30 days (Table 3). No significant difference in growth or survival of the F_1 generation was observed between the control and the three lower concentrations (16.8, 7.4, and 3.3 μg/liter).

Acute Exposure

Flow-through — The 96-hr TL50 and lethal threshold concentration (LTC) were determined for the three species based on measured concentrations. The results are listed in Table 4.

Static — The first group of fathead minnows placed in the static concentration of 550 μg/liter immediately after introducing the toxicant died within 8 hr. The second group placed in the same chamber 3 hr later lived without any apparent effects for the 10-day exposure. In a similar study a single

group of 10 was exposed to a 500-µg/liter concentration. Six of 10 died within 7 hr when exposed immediately after introducing the captan. All displayed the sympton of feeble swimming at the water surface that Holland et al. (1960) described for rainbow trout. The four that did not die appeared to recover and returned to their normal swimming habits for the duration of the 10-day exposure.

Discussion

Growth and survival of fathead minnows were not adversely affected by chronic exposure to 3.3, 7.4, and 16.8 µg/liter of captan. Postmortem examinations gave no indication that the gonads were immature or abnormal in any of the fish in the 7.4-µg/liter chamber where spawning did not occur. Perhaps the large ratio of males to females (Table 1) resulted in a disruption of normal spawning behavior. Nevertheless, the absence of spawning was not considered to be due to the effects of captan because spawning, although reduced, did occur in the higher concentration chambers. An adverse effect on mean spawnings/female and mean eggs spawned/female is suspected when the results from 39.5 and 16.8 µg/liter are compared to those from the control, 3.3 µg/liter and the 7.4-µg/liter chamber in which spawning did take place.

Statistical evaluation of the data indicates that the maximum acceptable toxicant concentration (MATC), as defined by Mount and Stephan (1967), lies between 39.5 and 16.8 µg/liter, based on survival rate and growth. An application factor (AF) defined by Mount and Stephan (1967) is obtained by dividing the MATC value by the 96-hr TL50. Eaton (1970) suggested that the lethal threshold concentration (LTC) is a better measure of acute toxicity than the 96-hr TL50 and modified the application factor to MATC/LTC. The application factor based on the fathead minnow MATC and LTC lies between 0.26 and 0.62. The estimated MATC (AF × LTC) is between 44.6 and 18.7 µg/liter for bluegills and between 18.0 and 7.5 µg/liter for brook trout.

Abedi and McKinley (1967) observed a dramatic eye and head injury in larval zebrafish exposed to 1.0 ppm of captan. Such injury was not found in 1-day-old fathead minnow larvae killed in the high captan concentration (63.5 µg/liter), nor was it observed in fathead minnow larvae exposed to 1 mg/liter captan. However, this may be due to species differences, because zebrafish larvae exposed to 1 mg/liter of captan in Lake Superior water developed eye damage similar to, but less severe than that reported by Abedi and McKinley (1967).

Captan hydrolyzes in water to tetrahydrophthalimide and tetrahydrophthalic acid; the rate of

TABLE 1. Survival and growth of parental fathead minnows (*Pimephales promelas*) after 51 days and 45 weeks of exposure to various captan concentrations.

	Measured captan concentration (µg/liter)											
	63.5		39.5		16.8		7.4		3.3		0 (control)	
Item	A	B	A	B	A	B	A	B	A	B	A	B
					51 days							
Survival (%)	4 *	0	80	72	84	88	92	84	76	72	88	80
Total length (*mm*)												
Mean	–	–	21.3 *	20.2	21.6	22.0	21.6	22.0	23.4	22.1	23.0	22.4
Range	–	–	28–16	30–13	29–14	32–13	34–13	28–13	33–19	31–14	33–10	30–12
					45 weeks							
Survival (%)	0 *	0	73.3	80.0	100	92.3	100	100	100	90.9	100	90.9
Males/females at termination	–	–	5/6	6/7	6/5	5/7	6/4	5/6	6/5	3/7	5/8	4/6
Mean total length (*mm*)												
Male	–	–	57.4 *	63.7	64.5	67.4	69.2	65.2	61.2	63.3	64.2	69.3
Female	–	–	50.0 *	51.4	50.6	54.9	55.5	57.0	59.2	54.4	58.1	58.5
Mean weight (*g*)												
Male	–	–	2.4	3.2	3.2	3.7	4.0	3.2	2.5	3.1	2.8	3.8
Female	–	–	1.3	1.4	1.3	1.5	1.6	1.7	1.7	1.4	1.7	1.8

*Values are significantly different from control values according to Dunnett's procedure ($P=0.05$).

TABLE 2. Reproductive success and embryo viability in fathead minnows exposed to various captan concentrations.

Item	Measured captan concentration (μg/liter)											
	63.5		39.5		16.8		7.4		3.3		0 (control)	
	A	B	A	B	A	B	A	B	A	B	A	B
Mean spawnings/female	–	–	0.14	0.71	2.4	1.9	0	12.7	10.4	4.6	14.6	9.6
Mean eggs spawned/female	–	–	68	12	485	360	0	1590	1648	400	1533	2173
Mean eggs/spawning	–	–	94.8	83.0	194	202	–	126	159	87	159	148
Hatchability (%)[a]	72.7[b]	66.4[b]	86.0	81.0	91.5	84.8	81.0[c]	87.1	71.6	77.7	76.6	68.1
Number of hatchability samples	3	5	1	2	8	8	8	15	15	12	18	20

[a]Each hatchability sample contained 50 1-day-old embryos.
[b]Control embryos incubated in 63.5 μg/liter.
[c]Embryos incubated in 7.4 μg/liter (A) from 7.4 μg/liter (B).

hydrolysis increases as the *p*H and temperature increase (J. N. Ospenson and D. E. Pack, Chevron Chemical Company, Richmond, Calif., *personal communication*). Captan's half-life in Lake Superior water with a *p*H of 7.6 is about 7 hr at 12 C and about 1 hr at 25 C. The difference between the nominal and measured concentrations in the chronic bioassay was due to the short half-life of captan in the test water. After subtracting the 12% captan that precipitated at the outlet of the needle, the measured concentrations in all test chambers averaged about 30% of the adjusted nominal concentrations. If a constant breakdown rate (a 1-hr half-life) is assumed, calculated test-chamber concentrations would be about 38% of the adjusted nominal concentrations. The difference between the calculated and measured test-chamber concentrations may have been due to small changes in the breakdown rate brought about by small changes in *p*H or temperature. Biodegradation may also have contributed to the breakdown rate especially between the 4th and 17th week when the concentrations failed to reach equilibrium.

Our static bioassays demonstrated that the breakdown of captan is rapid in Lake Superior test water and that the breakdown products from 550 µg/liter captan are not lethal to 3-month-old fathead minnows. Higher levels were not tested. Also, this fish is able to recover from sublethal effects that occur during acute exposures. Toxicity values that are based on initial (nominal) concentrations in static acute exposures are probably much higher than the true toxicity values because of captan's instability in water.

McKee and Wolf (1963) state that only 5% of the waters in the United States that support a good fish fauna have a *p*H less than 6.7. The half-life of captan in Lake Superior water when the *p*H is adjusted to 6.5 is about 8 hr at 23 C and about 40 hr at 12 C. Colder waters will increase its stability,

TABLE 3. Survival and growth of 30-day-old F_1 fathead minnows at various captan concentrations.

	Measured captan concentration (µg/liter)											
	63.5		39.5		16.8		7.4		3.3		0 (control)	
Item	A	B	A	B	A	B	A	B	A	B	A	B
Survival (%)	0[a] *	0[a]	27.5 *	33.8	65.0	61.3	45.0	65.6	47.5	72.5	69.4	72.5
Total length (*mm*)												
Mean	–	–	13.8 *	12.3	14.6	14.9	13.9	16.1	14.2	15.8	15.4	16.9
Range	–	–	25–10	20–7	24–9	24–8	24–7	29–6	27–8	30–7	26–8	28–11
Number of groups[b]	4	4	2[c]	4[d]	4	4	4	4	4	4	4	4

[a]Larvae in 63.5 µg/liter were from unexposed parents.
[b]Each group contained 40 1-day-old larvae.
[c]One group of larvae from control embryos incubated in 39.5-µg/liter concentration.
[d]Two groups of larvae from control embryos incubated in 39.5-µg/liter concentration.
*Values are significantly different from control values according to Dunnett's procedure ($P=0.05$).

TABLE 4. Captan acute toxicity, as measured by 96-hr TL50 and lethal threshold concentration (LTC), for three species of fish in continuous-flow exposures.

Species	Mean temperature (and range) (*C*)	Duration of experiment (*days*)	96-hr TL50 (and 95% CI) (*µg/liter*)	LTC (and 95% CI) (*µg/liter*)
Fathead minnow	25.2 (25.1–25.4)	6	65 (59–72)	64 (58–70)
Bluegill	24.9 (24.8–25.0)	5	72 (47–111)	72 (47–111)
Brook trout	11.8 (11.5–12.0)	8	34 (22–52)	29 (18–46)

but captan will likely break down rapidly in most warm surface waters supporting fish populations to form less toxic or nontoxic compounds.

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