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Effects of Chloroform, Tetrachloroethylene, and Trichloroethylene on Survival, Growth, and Liver of *Poecilia sphenops*

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A long-term study to observe the effects of chloroform, tetrachlorethylene and trichlorethylene on Poecilia sphenops, (the black molly) demonstrated adverse effects to growth, survival and liver morphology. These chemicals are common industrial compounds and are often released as waste, contributing to the pollutants of drinking water. Investigation of the deleterious effects of water pollutants is essential in determining the viability of fish and wildlife. The results of this study showed significant decrease in weight of the test animals, severe fatty change of the liver and high mortality during the 60-day test period.

METHODS

Adult black mollies, Poecilia sphenops, were used as test fish. All fish were acclimated for 90 days prior to testing. Six fish, three males and three females, were introduced into 16-L aquaria. Water for the aquaria was charcoal-filtered and then aged and aerated for ten days prior to the test period. 14L:10D photoperiods were maintained by artificial lighting. Water in the tanks was changed completely every two weeks. No charcoal filtering or floss filtering was used after the initial filtration of the water in order to prevent loss of the test chemicals. Fish were fed Tetra-Min Conditioning Food ad lib.

Seven groups of animals were maintained for a 60-day test period: 0.005 mL/L chloroform; 0.005 mL/L tetrachlorethylene; 0.005 mL/L trichlorethylene; 0.001 mL/L chloroform, 0.001 mL/L tetrachlorethylene and 0.001 mL/L trichlorethylene; and a control group. It should be noted that, due to the high volatility of these chemicals, final concentration of the chemicals before the two-week water changing probably was less than the initial concentration. Fresh solutions of the chemicals were prepared each time the water was changed.

Aquaria were checked several times daily for the presence of distressed or dead fish. (Deaths which would have ocurred between 9 PM and 7 AM would have resulted in omission of that test fish from the experiment. However, all deaths ocurred during the observation period of 7 AM to 9 PM, and all fish were removed within 2 h of death.) Distress was indicated by inability to swim, inability to respond to tapping on the aquaria or inability to

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navigate toward food. Severely distressed or dead fish were removed, weighed, and preserved in paraformaldehyde. Fish livers were removed and sectioned by frozen technique and stained with Oil Red O and Hematoxylin Eosin for histological analysis.

RESULTS

Mean weights and their standard errors for each test group are summarized in Table 1. Each of the test groups exposed to a test chemical showed a decline in weight during the 60-day test period. The control group increased in weight during the test period.

Table1. Initial and final mean weights and standard errors of Poecilia sphenops esposed to chloroform, tetrachlorethylene, and trichlorethylene for 60 days and control fish. (SE = standard error)

	initial mean wt.	SE	final mean wt.	SE
control group				
females	0.93	0.10	1.40	0.10
males	0.42	0.05	0.88	0.08
0.005 mL/L chloroform				
females	0.89	0.12	0.65	0.04
males	0.56	0.04	0.32	0.04
0.001 mL/L chloroform				
females	0.83	0.11	0.43	0.04
males	0.37	0.04	0.26	0.03
0.005 mL/L tetrachlorethylene				
females	0.99	0.03	0.61	0.05
males	0.55	0.08	0.37	0.07
0.001 mL/L tetrachlorethylene				
females	0.93	0.05	0.55	0.05
males	0.52	0.08	0.29	0.05
0.005 mL/L trichlorethylene				
females	0.78	0.07	0.51	0.09
males	0.33	0.05	0.28	0.04
0.001 mL/L trichlorethylene				
females	0.84	0.03	0.54	0.08
males	0.43	0.02	0.29	0.03

The test chemicals also had a major effect on survival of Poecilia sphenops. At the end of the 60-day test period, 31 of 36 test animals had died or appeared severely distressed and were removed. None of the 6 control animals appeared distressed or died during the test period. Data on survival are summarized in Table 2.

Histological analysis of liver tissue revealed considerable fat accumulation and some granuloma formation among the fish exposed to the test chemicals. These livers demonstrated large areas of tissue deterioration resulting in large open spaces in the tissue. The livers of the control fish exhibited scattered, tiny fat droplets and no large open spaces in the tissue. See figures 1 and 2.

Table 2. Survival time and sex of Poecilia sphenops exposed to chloroform, tetrachlorethylene, and trichlorethylene and control fish. (numerals indicate the order in which fish exposed to each chemical died or were removed; s = survived test period)

	sex	day removed
control group		
1	f	s
2	f	s
3	f	s
4	m	s
5	m	s
6	m	s
0.005 mL/L chloroform		
1	f	9
2	m	16
3	m	20
5	f	38
6	m	39
10	f	59
0.001 mL/L chloroform		
4	f	26
7	f	40
8	m	47
9	f	52
11	m	s
12	m	s
0.005 mL/L tetrachlorethylene		
1	f	5
2	f	11
3	m	13
5	f	17
10	m	34
11	m	59
0.001 mL/L tetrachlorethylene		
4	m	16
6	m	17
7	f	18
8	f	18
9	f	32
12	m	s
0.005 mL/L trichlorethylene		
1	f	9
3	m	23
4	f	27
5	m	34
10	m	59
11	f	s
0.001 mL/L trichlorethylene		
2	m	20
6	f	34
7	f	42
8	m	57
9	f	58
12	m	s

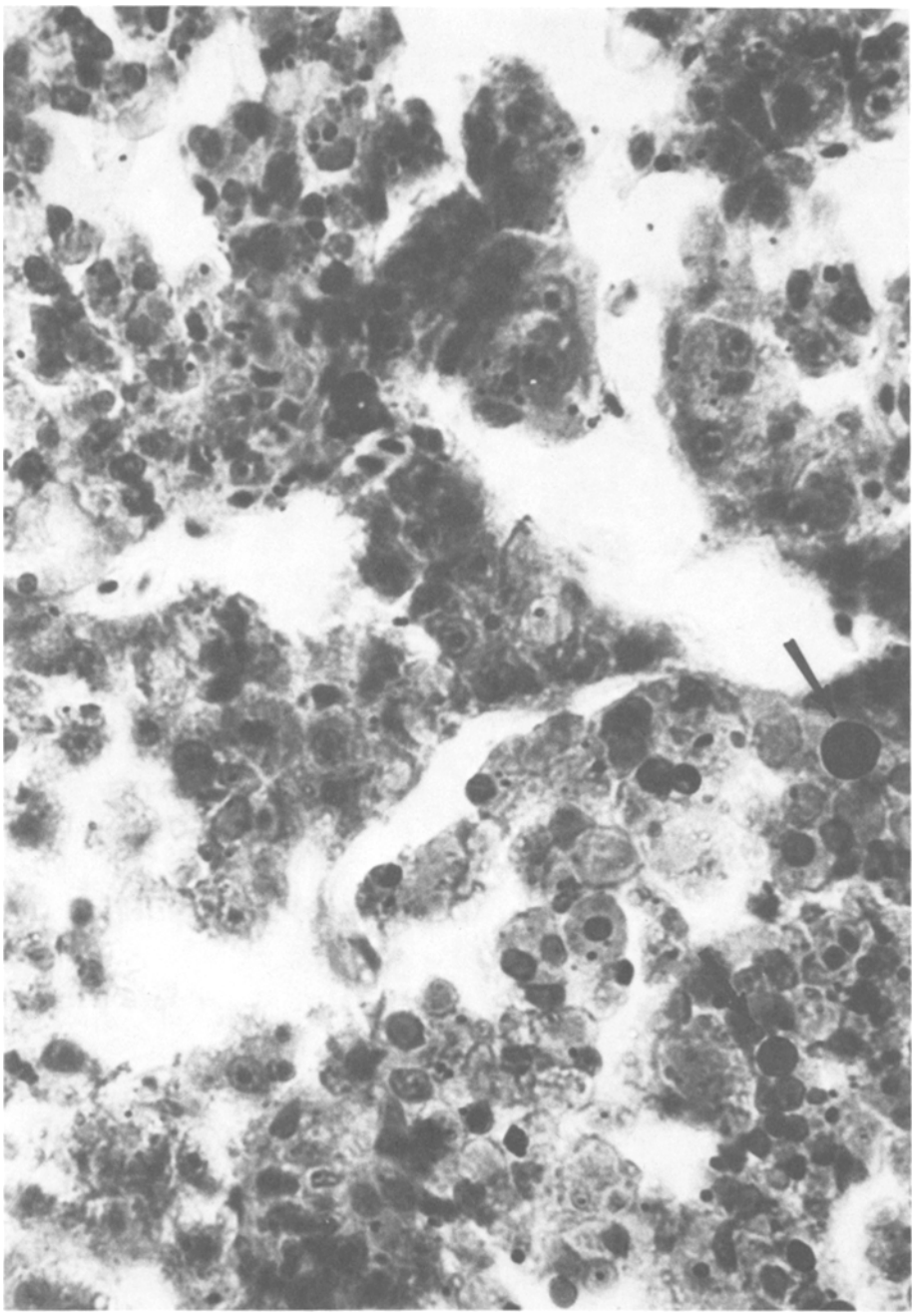

Figure 1. Poecilia sphenops exposed to chloroform for 40 days. Liver, stained with Oil Red O. Large fat droplets (arrows) and areas of tissue deterioration.

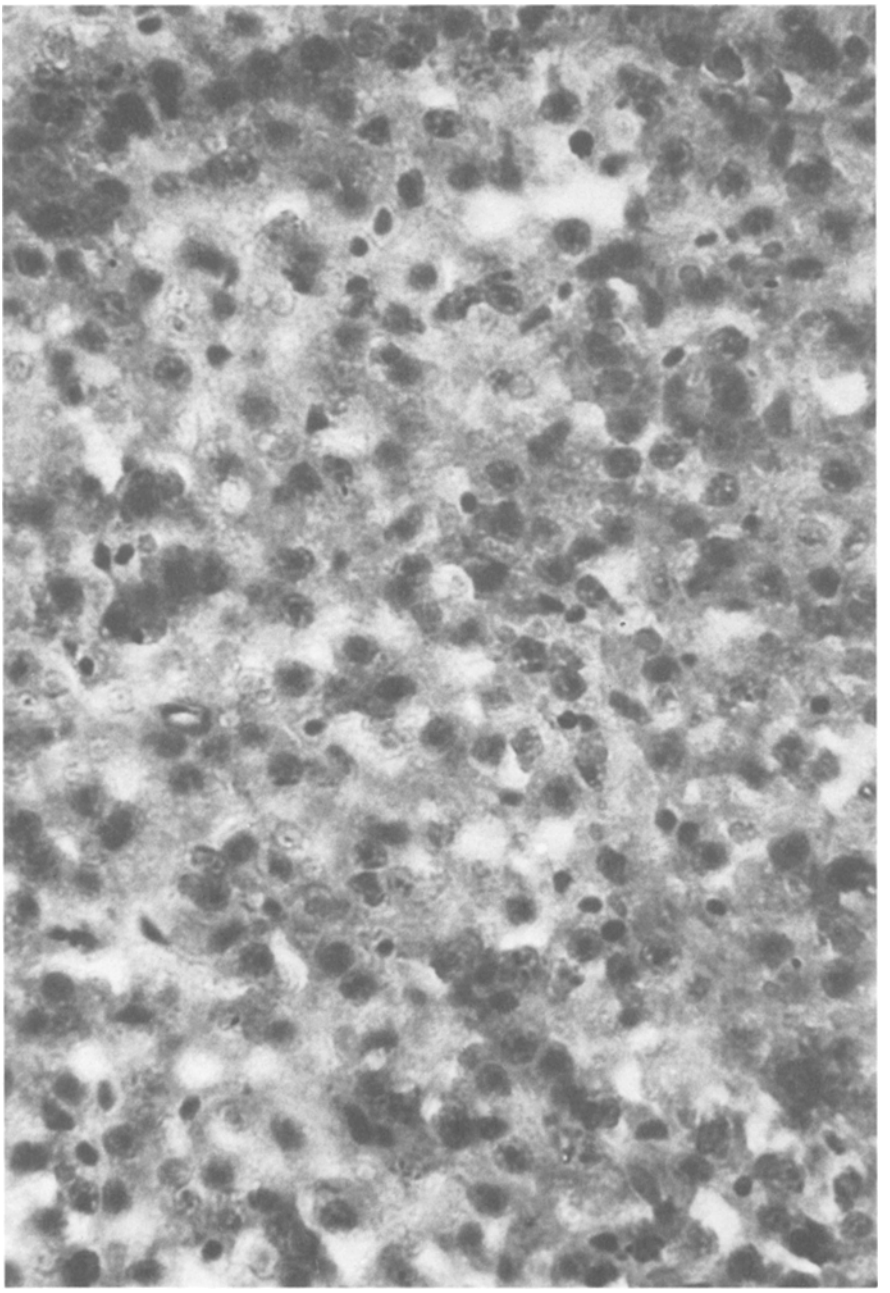

Figure 2. Poecilia sphenops, control group. Liver, stained with Oil Red O. Normal tissue morphology.

DISCUSSION

Previous researchers have studied the short-term effects of organochlorine compounds on various species of fish or have studied lethal doses of the chemicals (ALEXANDER et al. 1978; HENDERSON et al. 1979; STOCK & COPE 1969). However, very few researchers have studied long-term sublethal effects of organochlorine compounds (CRANDALL & GOODNIGHT 1962), and in addition to survival, parameters need to be studied analyzing the effect of organochlorine compounds among fish.

Survival was significantly affected by exposure to chloroform, tetrachlorethylene, and trichlorethylene. 31 of 36 test fish, or 86%, died or appeared severely distressed during the 60-day test period as compared to all of the control animals surviving without observable stress during the experiment.

In addition, growth, as indicated by total fish weight, was considerably reduced among the test animals during the test period. Each group of chemically-exposed animals had an average weight loss of 0.17 g among males, or 34.8% of initial total weight, and an average of 0.33 g among females, 37.5% of initial total weight. Several explanations are proposed for this phenomenon. Firstly, alteration of swimming ability could result in decreased ability to obtain food. Also, the chemicals may have an effect on metabolic function, specifically on food digestion, and food ingested may not have been converted to usable nutrients. Lastly, assuming food could have been ingested and digested in the presence of the chemicals, it seems very likely that food stored as fat in the liver may not be able to be mobilized, thus resulting in fat accumulation in the liver.

Finally, chloroform, tetrachlorethylene, and trichlorethylene demonstrated a striking change in liver morphology. The deterioration and open spaces observed in the chemical-exposed fish livers are, at least partially, due to increased fat accumulation. Other factors involved in this change need to be ingestigated further. Studies are presently underway to determine the mechanisms of weight loss and alteration of liver histology incurred from exposure to the chemicals. These studies include analysis of the liver for fat and glycogen and a longer term, lower dosage experiment with animals being removed at a set time schedule to analyze the effect of the organochlorine compounds with respect to time.

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