Acute Toxicity of Phosphate Ester Mixtures to Invertebrates and Fish

Michael J. Nevins and W. Waynon Johnson
U.S. Fish and Wildlife Service
Fish-Pesticide Research Laboratory
Columbia, Mo. 65201

The ubiquity of polychlorinated biphenyls (PCB's) RISEBROUGH and DE LAPPE 1972) and the discovery of their rates and routes of environmental transport (NISBET and SAROFIN 1972) have become an issue of major concern (ANONYMOUS 1976). Adverse environmental effects caused by PCB's (GUSTAFSON 1970, PEAKALL and LINCER 1970, RISEBROUGH et al. 1968, ZITKO and CHOI 1971) have led to an active search for more acceptable replacements. One group of candidates to replace PCB's in many applications are the phosphate esters, which are already used as lubricants, oil additives, plasticizers, and hydraulic fluids (SARACENO 1967, HEAPS 1968, ABADIR and MAYBERRY 1970). Total production of these phosphate esters in 1973 alone was 97 million pounds (LAPP 1976).

Commercial phosphate esters are complex mixtures of aryl and alkyl aryl phosphates that compose oily, viscous liquids with good thermal stability and resistance to oxidation (E.F. HOUGHTON and CO. 1973, MONSANTO INDUSTRIAL CHEMICAL CO. 1972). WAGEMAN et al. (1974) and LAPP (1976) reviewed the manufacture, composition, and use of these chemicals.

Toxicity of the phosphate esters to fowl, cats, dogs, and rats was investigated by BONDY et al. (1973), who found that the chemicals depressed plasma cholinesterase. The phosphates are neurotoxic to birds and mammals (FEST and SCHMIDT 1973) and have caused human poisoning by accidental ingestion (SAX 1968).

Information on the toxicity of phosphate esters to aquatic organisms is limited. WAGEMAN et al. (1974) reported that a triaryl phosphate, IMOL S-140, was not acutely toxic to rainbow trout (Salmo gairdneri) or guppies (Peocilia reticulata) during short exposures. However, a 4-mo exposure of rainbow trout to 0.3 to 0.9 mg/l of the phosphate ester mixture caused reduced feeding, discoloration of tissues, and finally death. In contrast to the observations of BONDY et al. (1973) on warm blooded animals, the rainbow trout did not show significant cholinesterase reduction.

The purpose of this study was to determine the acute and sub-acute toxicity of phosphate ester hydraulic fluids to freshwater invertebrates and fish. Three hydraulic fluids were tested:

Bulletin of Environmental Contamination & Toxicology,
0007-4861/78/0019-0250 $1.40

Houghtosafe 1120, manufactured by E.F. Houghton and Co.[1], and Pydraul 50E and Pydraul 115E, manufactured by Monsanto Chemicao Co. They are considered typical representatives of industrially used hydraulic fluids.

MATERIALS AND METHODS

Invertebrates used in toxicity tests were mature scuds (Gammarus pseudolimnaeus) from laboratory cultures and crayfish (Orconectes nais) collected from local ponds. Fish used in toxicity tests were rainbow trout (Salmo gairdneri), fathead minnows (Pimephales promelas), channel catfish (Ictalurus punctatus), and bluegills (Lepomis macrochirus) from National Fish Hatcheries. All test animals were held in the laboratory for a minimum of 10 days and acclimated to test conditions for 4 days before use (BRAUHN and SCHOETTGER 1975, COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS 1975).

Methods for static and flow-through toxicity tests were those recommended for standardized laboratory toxicity tests (COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS 1975). Static tests with fish were conducted in reconstituted soft water (hardness 44 mg/l, alkalinity 35 mg/l, pH 7.4); static tests with invertebrates and all flow-through tests were conducted in well water (total hardness 272 mg/l, alkalinity 237 mg/l, pH 7.4) sterilized under UV light.

Flow-through tests were conducted with a proportional diluter (MOUNT and BRUNGS 1967); 41-liter glass aquaria were used as exposure chambers and the toxicant was metered with a device designed by McALLISTER et al. (1972). Static tests were conducted in 15-liter glass jars. The concentration of the acetone used as a solvent for the toxicant did not exceed 0.67 ml/l in static tests or 0.20 ml/l in flow-through tests. Ten organisms per concentration were used in static tests and 20 per concentration in flow-through tests; at least eight concentrations were used in each test.

Test organisms were not fed during static tests nor during the first 4 days of flow-through tests. Beginning with the 5th day, fish in flow-through tests were fed to satiation once daily with Ewos trout food. Observations were recorded and dead organisms were removed from test containers daily.

The median lethal concentration (LC50) and its 95% confidence limits were calculated by the method of LITCHFIELD and WILCOXON (1949). In tests where less than half of the organisms died in the highest exposure, the LC50 was stated as being greater

[1]Reference to trade names does not constitute government endorsement of commercial products.

than the highest concentration. Flow-through tests were continued for 30 days or until the total daily mortality declined to 10% or less of the number of animals per concentration, for a minimum of 5 consecutive days. An LC50 was calculated for alternate days after the daily mortality rate reached 10%. Using these data, we calculated the time independent median lethal concentration (TILC50) by the method of GREEN (1965). Any two LC50's were considered significantly different when the 95% confidence intervals about the LC50's did not overlap.

RESULTS AND DISCUSSION

Pydraul 50E was significantly more toxic than Houghtosafe 1120 to fathead minnows, channel catfish, and bluegills in static and flow-through tests. The 96-h LC50's for Houghtosafe 1120 were 5 to 27 times greater than those for Pydraul 50E in static tests (Table 1), and 4 to 8 times greater in flow-through tests

TABLE 1

The acute toxicity of tri-aryl phosphate hydraulic fluids in static tests.

Hydraulic fluid and species	Temp (°C)	LC50 (mg/l) and 95% confidence limits	
		24 h	96 h
Houghtosafe 1120			
Scud	20	3.1 (2.3-4.2)	0.70 (0.53-0.92)
Rainbow trout	10	4.2 (3.0-5.8)	1.7 (1.2-2.5)
Fathead minnow	17	>90	35 (24-49)
Channel catfish	20	130 (87-190)	43 (31-59)
Bluegill	20	32 (18-57)	12 (8.0-19)
Pydraul 50E			
Scud	20	1.5 (1.2-2.0)	0.56 (0.40-0.78)
Rainbow trout	10	1.3 (0.88-1.8)	0.72 (0.52-1.0)
Fathead minnow	20	2.5 (1.1-5.5)	1.3 (0.49-3.2)
Channel catfish	20	7.2 (5.5-9.5)	3.0 (2.2-4.1)
Bluegill	20	4.4 (3.0-6.3)	2.2 (1.5-3.2)
Pydraul 115E			
Rainbow trout	10	>100	45 (34-60)
Channel catfish	20	>100	>100
Bluegill	20	>100	>100

(Table 2). Scuds and rainbow trout, the most sensitive species exposed, had 96 h LC50's for both compounds ranging from 0.56 to 1.7 mg/l. Crayfish showed no apparent response to Pydraul 50E after 20 days of flow-through exposure at concentrations up to 1.5 mg/l.

STALLING and MAYER (1972) reported a 96-h LC50 of the PCB Aroclor 1242 to cutthroat trout (*Salmo clarki*) in static tests as 5.43 mg/l. Rainbow trout (which represented the salmonids in the present study) were less sensitive to Pydraul 115E (45 mg/l), more sensitive to Houghtosafe 1120 (1.7 mg/l) and much more sensitive to Pydraul 50E (0.72 mg/l) than were cutthroat trout to Aroclor 1242. STALLING and MAYER (1972) also exposed rainbow trout, channel catfish, and bluegills to four PCB's by the flow-through method. The LC50's at the end of their tests, 10 to 30 days, ranged from 0.008 to 0.151 mg/l. The range of end-of-test LC50's for Houghtosafe 1120 and Pydraul 50E for the same species was generally higher, 0.37 to 5.0 mg/l, indicating that these phosphate esters are less acutely toxic to fish in flow-through test than are PCB's. Static tests indicated that scuds are more susceptible to both Houghtosafe 1120 (96-h LC50, 0.7 mg/l) and Pydraul 50E (0.56 mg/l) than to Aroclor 1254 (2.4 mg/l), but less susceptible to these two phosphates than to Aroclor 1248 (96-h LC50, 0.052 mg/l).

Sublethal reactions to Houghtosafe 1120 and Pydraul 50E were noted in most toxicity tests, although the effects were more pronounced in the longer flow-through tests. Fathead minnows, channel catfish, and bluegills fed less, became hypersensitive to disturbance, developed hemorrhagic areas around the dorsal fin, and exhibited impaired swimming ability, even when mortality in the test was low. Hemorrhaging in the dorsal fin area and impaired swimming were especially evident in bluegills. These symptoms could be the result of toxicant-induced vertebral damage such as McCANN and JASPER (1972) observed in bluegills exposed to organophosphate pesticides. Subacute effects were present at concentrations as low as one half of the LC50's. Some affected fish did not die by the end of the test, but none showed signs of recovery. Rainbow trout, the most sensitive fish species, showed few or no subacute effects.

The TILC50 data (Table 2) indicate that concentrations of hydraulic fluids as low as 0.25 mg/l are harmful to fish. This observation agrees with the findings of WAGEMEN et al. (1975) on IMOL S-140 (0.3-0.9 mg/l). If only the acute toxicity data are considered, the phosphate esters as replacements for PCB's in hydraulic fluids may reduce somewhat the toxicity hazard of such materials to aquatic organisms. However, the sublethal reactions of fish to the phosphates strongly suggest a need for further studies on their chronic toxicity to fish and aquatic invertebrates and on the persistence of these chemicals in aquatic habitats.

TABLE 2

The acute and sub-acute toxicity of tri-aryl phosphate hydraulic fluids in flow-through toxicity tests.

Hydraulic Fluid and species	Temp (°C)	LC50 (mg/l) and 95% confidence limits: 96 h	LC50 (mg/l) and 95% confidence limits: End of test	TILC50[a]	Test duration (days)
Houghtosafe 1120					
Rainbow trout	17	0.65 (0.55-0.77)	0.59 (0.50-0.69	0.54	8
Fathead minnow	17	17 (14-21)	8.5 (7.3-9.9)	6.6	20
Channel catfish	17	>15	4.5 (3.3-6.1)	ND	30
Bluegill	20	11 (9.1-12)	5.0 (4.4-5.7)	3.4	17
Pydraul 50E					
Crayfish	17	>1.5	>1.5	ND	20
Rainbow trout	17	0.67 (0.56-0.81)	0.37 (0.33-0.42)	0.25	15
Fathead minnow	17	2.1 (1.9-2.3)	1.7 (1.5-2.0)	1.5	10
Channel catfish	17	2.4 (2.2-2.6)	2.1 (1.9-2.3)	2.1	30
Bluegill	20	2.8 (2.5-3.1)	2.1 (1.9-2.2)	1.6	11

[a]TILC50 not determined for channel catfish in Houghtosafe 1120 because of high mortality or for crayfish in Pydraul 50E because no mortality occurred.

SUMMARY

The acute toxicity of three commercial phosphate ester mixtures (hydraulic fluids) was tested against two invertebrates and four fishes. Pydraul 50E was more toxic than Houghtosafe 1120 in static and flow-through tests; Pydraul 115E was the least toxic. In flow-through tests these phosphate esters were less acutely toxic than PCB's. However, sublethal reactions to the phosphates suggest a need for chronic toxicity studies.

ACKNOWLEDGMENTS

We thank Herman O. Sanders for conducting the tests with invertebrates and for constructive criticism of the manuscript.

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