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Toxicity of the Organophosphate Insecticide Fenthion, Alone and with Thermal Fog Carriers, to an Estuarine Copepod and Young Fish

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Fenthion (O,O-dimethyl O-[3-methyl-4-methylthio-phenyl] thiophosphate; Mobay Chemical Corp., Kansas City) is an organophosphate insecticide registered as Baytex® that is used in the hygiene sector for control of flies, mosquitoes, ticks, and lice. Fenthion is also widely used in the control of agricultural pests and is recommended for control of parasites of economically important pond-cultured fish.

Fenthion often is applied aerially as a thermal fog over land adjacent to coastal waters and marshes for control of adult mosquitoes. Although fog formulations may vary, all are a mixture that is mostly fuel oil and a lesser amount of active ingredient (= AI); In Florida, the formulation (called Baytex thermal fog) is 65% diesel fuel, 33.5% fog oil of light naphthenics and 1.5% fenthion. Thermal fogs are not intentionally applied over water; however, accidental contamination of water may occur, either directly or by drift.

Results of field studies by van Dyk et al. (1975), Clarke et al. (1987), and Tucker et al. (1987) have shown that there is mortality among non-target aquatic arthropods following experimental applications of Baytex thermal fog.

Previous laboratory studies on the toxicity of Baytex to non-target marine and freshwater species have focused on fenthion alone (Korn and Earnest 1974; Kenaga 1979; Johnson and Finley 1980). Newell et al. (1981) studied the toxicity of fenthion to microbes and small invertebrates of a mangrove estuary using a fog mixture of fenthion and petroleum distillates as their source of fenthion. Oil controls were not used. Any role that oil carriers might play in Baytex fog toxicity in the laboratory or in the

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field has not been addressed, even though petroleum hydrocarbon mixtures (particularly fuel oils) are known to be toxic to a variety of marine organisms (Anderson et al. 1974).

In this study, a series of laboratory tests were performed to compare the acute toxicity of technical grade fenthion, Baytex fog (fenthion plus oils), and the oil carriers alone to a variety of non-target estuarine organisms. Toxicity tests were performed on hatching eggs and juveniles of the spotted seatrout (Cynoscion nebulosus) and the common snook (Centropomus undecimalis), hatching eggs of spot (Leiostomus xanthurus) and gulf menhaden (Brevoortia patronus), and adults of the calanoid copepod, Acartia tonsa. The fish are important in commercial and recreational fisheries. All species tested are ecologically important and occur in the water of potentially affected areas.

MATERIALS AND METHODS

Gulf menhaden and spot eggs were obtained by hormone-induced ovulation followed by voluntary tank spawning. Snook and seatrout eggs were obtained by hormone-induced ovulation and manual fertilization. Hatching rates of eggs obtained this way were between 95 and 100% for all but the snook whose hatching rate was 63%. Eggs were incubated in the laboratory until transfer to test chambers. Juvenile snook 30-50 mm long (fork length) were seined from the Indian River lagoon, Florida (27°25'N, 80°20'W). Juvenile seatrout 17-21 mm long (total length) were from cultured stocks. Copepods were collected from a bridge crossing the Indian River, with a 318 μm mesh plankton net and were held overnight under laboratory conditions prior to starting tests. A. tonsa have a strong photo-positive response which was used to separate them from other organisms: A light was placed at the top of the collection chamber and the copepods attracted to it were removed with a large bore pipette.

Technical grade fenthion (98%) was obtained from the manufacturer. Baytex thermal fog mixture and diesel fuel and fog oil for fog oil mixture were obtained from Lee County Mosquito Control District, Florida. The Baytex fog formulation was 98.5% oil mixture (65% diesel fuel + 33.5% light naphthenics) and 1.5% fenthion. Toxicant stock solutions were prepared by mixing toxicant in the carrier solvent acetone. Indian River water was used for toxicant/saltwater mixtures. Before tests, it was filtered through sand and activated charcoal and UV-sterilized. If necessary, salinity was adjusted by addition of reagent grade NaCl or by fresh-water dilution. The pH was between 7.7 and 8.2. Test toxicant solutions were prepared by adding 100 μL of the appropriate stock

solution to each liter of saltwater followed by vigorous mixing. Resulting carrier solvent concentrations were thus $\leq$ 100 μL/L. Controls contained 100 μL acetone/L test mixture.

Animals were exposed at constant temperature ($\pm$ 1.0 °C, Table 1, next page) to one of 6 to 12 toxicant concentrations. Temperatures and salinities used for fish eggs were optimal for development, and those used for juvenile fish and copepods were near ambient at the time and place of capture. Exposures were static with constant indirect dim light for 48 h. No food was provided during tests. Fish eggs (20/replicate) were tested in glass crystallizing dishes containing 240 mL test solution. Larger dishes containing 1.5 L test solution were used with juvenile fish (5/replicate). Copepods (mean = 50/replicate) were tested in small dishes containing 100 mL test solution. All tests had three replicates per treatment.

Every 12 h, dead animals were removed and counted. Eggs and larvae were counted separately in tests with hatching fish eggs. Estimates of LC50s and 95% confidence limits were made using the graphical log-probit method of Litchfield and Wilcoxon (1949) or binomial probability analysis. Acute lethality values for tests with hatching fish eggs are for those that hatched (larvae); preliminary analysis of variance indicated that hatching rates in groups exposed to toxicant were not significantly different from the mean (95-100%) observed in controls ($p > 0.75$). Differences between the toxicity of Baytex fog and its components were identified by simple comparison of LC50s and 95% confidence limits. Results of tests with sufficient data were analyzed for additivity by calculating additive indices and their ranges from LC50s and 95% confidence limits (Marking 1984).

The concentration of fenthion in exposure mixtures was measured at the start of each test. Subsurface water samples were extracted with petroleum ether. Extracts were analyzed with a Hewlett-Packard 5730A gas chromatograph equipped with an N-P detector. Laboratory quality was checked by analyzing extra samples with internal spikes each day that toxicant solutions were analyzed. The detection limit was 0.01 ng fenthion/mL and recovery efficiency 90-112%. The amount of oil in Baytex fog solutions and corresponding oil mixture controls was computed from measured concentrations of fenthion.

RESULTS AND DISCUSSION

Fenthion alone was toxic at higher concentrations than was fenthion in fog mixture (Table 1). The 48-h LC50s for technical grade fenthion were greater than 300 ng/mL

Table 1. Toxicity of Baytex fog (fenthion + oil mixture), technical grade fenthion, and oil mixture.

Organism	Temp. (°C)	Sal. (°/oo)	48-h LC50 (95% Confidence Limits), ng/mL			
			Baytex fog[2]		Fenthion only	Oils only
Seatrout eggs	26	30	Fenthion	5 (4-5)	1,333 (1,162-1,530)	
			Oils	248 (227-269)		432 (339-554)
Seatrout juveniles	26	36	Fenthion	8 (5-15)	1,130 (1,023-1,249)	
			Oils	432 (243-775)		-
Snook eggs	28	33	Fenthion	54 (19-88)	1,370 (973-1,765)	
			Oils	2,848 (1,002-4,641)		2,848 (1,002-4,641)
Snook juveniles	23	22	Fenthion	625 (420-830)	1,015 (430-2,600)	
			Oils	32,963 (22,151-43,774)		-
Spot eggs	20	30	Fenthion	48 (34-67)	1,501 (988-2,014)	
			Oils	2,532 (1,793-3,534)		-
Menhaden eggs	20	30	Fenthion	115 (94-140)	540 (483-604)	
			Oils	6,065 (4,958-7,384)		3,480 (1,851-6,540)
Copepod adults[1]	25	31	Fenthion	14 (11-16)	830 (772-892)	
			Oils	712 (603-840)		580 (488-680)
Copepod adults	24	29	Fenthion	<28	550 (367-825)	
			Oils	<1477		-

[1] 24-h LC50s. Approximate values for 48-h LC50s are; 1) Baytex fog = 2.3 ng/mL fenthion + 121 ng/mL oils, 2) oil mixture only = 121 ng/mL and, 3) fenthion only = 470 ng/mL

[2] Additive index (and range): 0.74 (0.25 to 1.42) for seatrout eggs, -0.04 (-3.72 to 3.41) for snook eggs, -0.96 (-3.28 to 0.32) for menhaden eggs, and -0.25 (-0.74 to 0.13) for adult copepods.

Control mortality: 10-15% for menhaden, spot and seatrout larvae, 27% for snook larvae and seatrout juveniles, 0% for snook juveniles, 20-25% for adult copepods.

and in most cases above 1,000 ng/mL. The toxicity of technical grade fenthion to young spot, snook, and seatrout in this study was comparable to that reported for mullet (48-h LC50 = 1,600 ng/mL, Kenaga 1979). The slightly lower 48-h LC50 for menhaden eggs (Table 1) was also observed with young striped bass in studies by Korn and Earnest (1974, 48-h LC50 = 453 ng/mL).

Copepods were more sensitive to fenthion than most of the fish tested (Table 1). Like other organophosphates, fenthion is generally more toxic to crustaceans than to fish; however, it was considerably less toxic to Acartia tonsa than to other crustaceans. The 24-h LC50 for the mysid Mysidopsis bahia (0.42 ng/mL) and the grass shrimp Palaemonetes pugio (9.5 ng/mL, Clarke et al. 1987) and the 48-h LC50 for pink shrimp (Penaeus duorarum) (0.06 ng/mL, Kenaga 1979) are orders of magnitude lower than was observed in tests with Acartia tonsa (Table 1).

Baytex fog (relative to fenthion concentration) was always more toxic than fenthion alone (Table 1). The difference was significant for all animals tested except common snook juveniles (Table 1). Acutely toxic concentrations of Baytex fog were lowest for seatrout and copepods (48-h LC50s <10 ng/mL fenthion), intermediate for newly hatched snook and spot (48-h LC50s = 10-100 ng/mL fenthion), and highest for snook juveniles and hatching menhaden (48-h LC50s >100 ng/mL fenthion).

Lethality observed with Baytex fog was matched by that of the oil mixture controls; LC50s for oil alone were very near, if not the same as LC50s for oil in Baytex fog (Table 1). The lethality of oil mixture matched Baytex fog lethality better than fenthion did at all concentrations used (Figure 1, next page). Fenthion by itself did not cause significant mortality, compared with controls, until it reached levels greatly exceeding those in Baytex fog and oil mixtures that caused 100% mortality. For four of the organisms tested, the contribution of oil to Baytex fog toxicity was at least as great as suggested in Figure 1 and by LC50s in Table 1: The toxic action of fenthion and oil together was described by simple additivity (index range overlapped zero, Table 1) for menhaden eggs, snook eggs, and adult copepods, and was more than additive for seatrout eggs (index >0, Table 1). The type of toxicant interaction that occurred with other organisms was not determined; however, it appears that the toxicity of Baytex fog to non-target organisms in this study resulted mostly from the presence of oil.

The water soluble fraction of fuel oils is known to be acutely toxic to a variety of marine organisms: 48-h LC50s for adult subtropical representatives of common estuarine crustaceans and fish are between 900 and 5,000

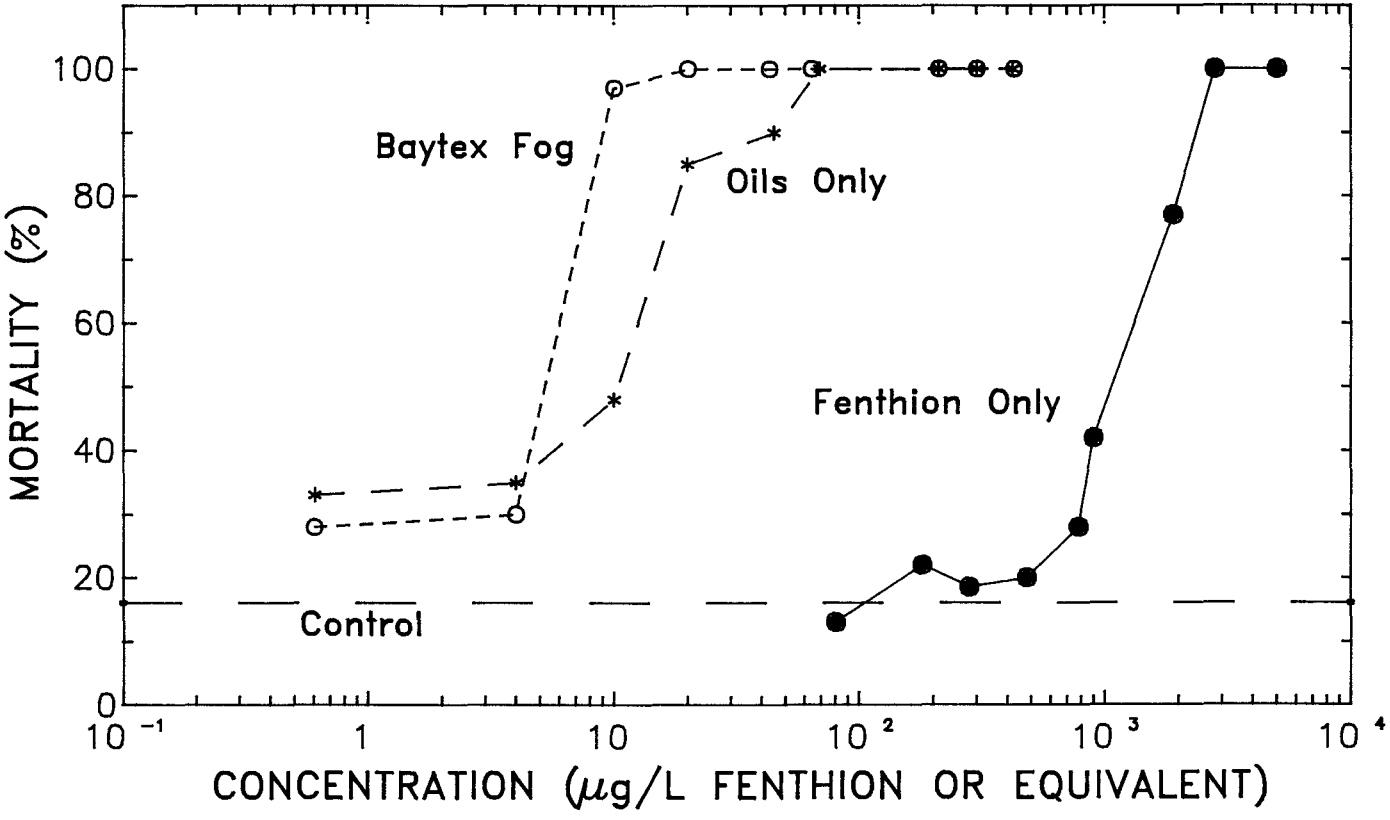

Figure 1. Mean mortality of seatrout larvae exposed to Baytex fog (fenthion + oils), technical grade fenthion and oil mixture controls, plotted in terms of fenthion concentration

ng/mL (Anderson et al. 1974). Johnson et al. (1979) found that 48-h exposure to 100 ng/mL of the water soluble fraction of fuel oil was not acutely lethal to spotted seatrout larvae but, at the end of the 48-h exposure, there was a reduction in growth and a quarter of the larvae had not developed eye pigmentation. It is possible that similar concentrations of diesel fuel were lethal for organisms in this study. Lethal oil concentrations were estimated from the amount added which ranged from 200 to 7,500 ng/mL in most cases. The concentration of oil that caused lethality had to be lower because diesel fuel was only 65% of the mixture. Furthermore, only a fraction of the amount of fuel oil added is water soluble and not all components of the water soluble fraction of fuel oils are toxic. Some toxicity of the Baytex fog mixture may be attributed to the fog oil which was a mixture of light naphthenic distillates. Separate tests on fog oil toxicity were not conducted.

Field tests in which Baytex fog was applied directly over water have resulted in mortality of non-target arthropods. On the lower-Orange river in South Africa, van Dyk et al. (1975) observed an immediate decline in the number of aquatic crustaceans and insects after a single application, followed by a decline downstream the next day. In five separate tests on the Gulf coast of Florida, Clarke et al. (1987) observed mortality among caged mysids and pink shrimp exposed to Baytex fog at one site (a residential canal) where flushing is probably limited but not at another site with better circulation (an estuarine bay). Tucker, et al. (1987) observed mortality among caged Acartia tonsa in one of four tests performed in a mosquito impoundment near Fort Pierce, Florida.

Mortality of non-target organisms in field studies with Baytex fog may be from exposure to fenthion, oil adjuvants, stressful environmental conditions in the field at the time of the test (e.g., high temperature and low dissolved oxygen, Tucker et al. 1987), or any combination of the three. The occurrence of fenthion in experimentally exposed waters was found to be short-term and rapidly changing; concentrations peaked then diminished within hours after direct application (van Dyk et al. 1975; Clarke et al. 1987; Tucker et al. 1987) or after transport from upstream (van Dyk et al. 1975). Peak fenthion concentration following a single direct application was 0.742 ng/mL (Van Dyk et al. 1975, using 20% AI) or ranged from not detectable to 2.6 ng/mL (Clarke et al. 1987, using 1.5% AI), or from $\leq$0.01 to 1.69 ng/mL (Tucker et al. 1987, using 1.5% AI). Fenthion toxicity might contribute to mortality in those tests where peak concentration exceeds 12- or 24-h LC50s (e.g., as for mysids and pink shrimp, Clarke et al. 1987). However, results of this study indicate that the oil adjuvants contribute significantly to Baytex fog toxicity

in the laboratory. Fog oil mixtures may also be largely responsible for mortality in the field. It is recommended that the role of fuel oil based fog carriers be considered in future field studies on insecticide toxicity.

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