Bull Environ Contam Toxicol (1984) 32:587–596
© 1984 Springer-Verlag New York Inc.

Environmental Contamination and Toxicology

Fish Mortality Following Application of Phenthoate to Florida Citrus

Herbert N. Nigg, James H. Stamper, Robert M. Queen, and Joseph L. Knapp

University of Florida, Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

Florida citrus growers regularly apply pesticides near bodies of water and also may pump water from nearby lakes and ditches to supply spray machines. Phenthoate (O,O-Dimethyl S-(α-ethoxycarbonylbenzyl)-phosphorodithioate) may be registered with the U.S. Environmental Protection Agency for use in Florida. Phenthoate is the active ingredient in Cidial. It is highly toxic to fish with a listed 96 h LC_{50} of 3.3 ppb (bluegill) (Mark Burt, Ph.D., Montedison, USA, personal communication). By comparison, ethion is commonly used in Florida citrus and has been implicated in several large fish kills in citrus areas (HN, personal observations). Its 96 h LC_{50} is reported as 210 ppb (bluegill) (U.S. Dept. of Interior, 1980). This experiment was conducted to determine if phenthoate would cause fish mortality under 'normal' Florida conditions.

MATERIALS AND METHODS

4E (4 lb/gal emulsifiable) phenthoate was applied on April 27, 1982 to 33 acres of mature citrus (Valencia, Pineapple and Walker earlies) trees at a rate of 10 lb phenthoate AI/A with 10.5 lb Cu/A, 1 lb Bo/A, 3 gal oil/A in 250 gal H_2O/A. The distance from the edge of the treatment pond to the nearest row of citrus trees was approximately 30 ft. Water for the spraying operation was pumped from the treatment pond. The supply vehicle was backed to the edge of the pond and a 4" hose inserted into a wire mesh 1' x 1' x 1' basket resting on the bottom of the pond. The top of the basket was out of the water. This is standard practice in many Florida citrus groves. Water is pumped into the supply vehicle and, as the vehicle is filling, the pesticides are added to the tank. When the tank is full, a valve is closed preventing the spray mixture from entering the hose and the pond, and the pump is shut off. Additionally, the hose is equipped with a footvalve. Mixer-loaders sometimes inadvertently overfill the tank and turn off the pump but do not close the rear valve. In this event, the footvalve prevents drainage of pesticide mixture into the pond. The footvalve in this study was in excellent condition, i.e., was not allowing leakage into the pond. Airblast equipment was used and the pond-side nozzles were turned off when trees immediately around the pond were sprayed. The wind was 3 to 5 mph, gusting to 8 mph, out of the west. Fish (bluegills) were distributed among three cages in each of two ponds: a 'treatment' pond in the sprayed citrus grove and a similar 'control' pond at considerable distance from any spraying activity.

Water samples to test for phenthoate contamination were taken from the treatment and control ponds, one from inside each of the three fish cages and one from the area nearby each cage, for a total of 6 samples/pond. Water samples were taken the day before and at 3 h, 1, 2, 3, 4, 5, 6, 7, 8 and 9 days postapplication. Samples were taken in hexane-washed 500 ml brown glass bottles with teflon-lined caps. All water samples were 500 ml and were individually extracted and analyzed. Water temperature and dissolved oxygen were taken 1 h before application and at 1, 2, 3, 4, 5, 6, 7 and 8 days postapplication.

Drift of airborne pesticide into the ponds was assessed by a series of 4" x 4" α-cellulose pads on top of 3 ft stakes placed at 10 ft intervals. At the treatment pond, there were two series of stakes: one surrounding the shoreline of the pond and another concentric series on higher terrain just off the shoreline. Each stake was assigned a number, starting at the boat launch area and proceeding around the pond in counter-clockwise ascending order. Pads were also placed on the fish cages diagonally across the top, three/cage. There was a total of 41 stakes in the inner (shoreline) series of the treatment pond and 50 stakes in the outer (slope's edge) series. At the control pond there was only one series of 16 stakes with α-cellulose pads, placed along the part of its shoreline closest to the treatment pond.

Pads were collected immediately after application: from the control pond first, to minimize contamination, then from the treatment pond. All pads collected were wrapped in aluminum foil, labeled, placed on ice and frozen at -20°C within 1/2 h until extraction and analysis.

The two ponds are located in central Polk County, Florida. The treatment pond is located at Township 27S, Range 26E, SW 1/4, NE 1/4, NW 1/4, Section 25, with a water surface area of approximately 0.25 A. This pond was formed by the natural collapse of the limestone which underlies much of Florida. The bank is sloped at an average inclination of approximately 20° during an 8 to 10 ft drop from the outer edge to the water's surface. The depth profile within the pond is a gentle slope from shoreline to 8 ft deep 20 ft from shore, then to a maximum of 10 ft at the pond's center, with an overall average depth of 8.5 ft. The shoreline is screened by sparse low-growing mixed shrubs. Objects could be seen to a depth of approximately 4 ft. The bottom was carpeted with miscellaneous aquatic vegetation. The predominant vegetation was a mixture of cattails (Tytha latifolia) and Maidenhead grass (Panicum hemitomon). The control pond appeared to be man-made and is located at Township 27S, Range 26E, SE 1/4, NE 1/4, Section 27. The pond has a surface area of approximately 0.77 A. The depth profile is a gentle slope from shoreline to 8 ft deep 20 ft from shore, to approximately 4 ft deep in the middle. The pond averaged 3.5 ft in depth. The water was dark due to runoff, the lack of any shrubbery on the shoreline, and the depth profile of the pond. The predominant vegetation in the pond was water lily (Nelumbo lutea). The pond is surrounded by approximately 200 A of citrus on the north, west and east. It is bordered on the northeast by a storage barn, and on the south by Seaboard Coast Line railroad track. The ponds were chosen for their proximity to citrus, to one another, and for the fact that both ponds were shown to contain a bluegill sunfish population.

The bluegills were obtained from Southern Fish Culturists, Leesburg, FL. Bluegills 1" to 2" in length were selected, transported and caged in both ponds 4 days prior to the application. The fish were precounted and placed in 6 plastic bags prior to being transported to the ponds. Fish were caged in 3' x 3' wood and screen mesh floating cages with a top door. The screening, XV1679, 7 x 5 strand count, was obtained from Donovan Enterprises, Inc., Palm City, FL.

Four days prior to application, 2 minnow traps with Purina Catfish Chow were placed in each of the ponds to determine if the ponds contained a bluegill population. Bluegills were found in both traps in the control pond. Only one of the traps in the treatment pond contained fish, but it contained 13 bluegills. The fish were counted and released.

Caged fish were fed daily by placing 10 to 15 Purina Catfish Chow pellets per cage on the water surface underneath the cage door. Dead fish were counted as they were removed from the cages with a dip net.

The 500 ml water samples to be analyzed for phenthoate contamination were extracted with 50 ml of methylene chloride followed by a 125 ml n-hexane extraction. Both extractions were combined, reduced to dryness on a rotary evaporator at 40°C and transferred 3X into 10 ml of isooctane for gas-liquid chromatography.

Analysis of the chemical characteristics of water from both ponds was performed by Mitco Water Laboratories, Inc., Winter Haven, FL. Samples were transported to the Mitco Water Lab on the day before application and on days 9, 14 and 28 postapplication. The water temperature was obtained by suspending a thermometer (-35 to 50°C, Science Associates, Princeton, NJ) in the water near the center of the pond. Dissolved oxygen was measured using the Yellow Springs Instrument (YSI) Model 54A oxygen analyzer powered by a 115V

Honda Generator, Model E1500.

The 4" x 4" α-cellulose pads were sectioned into quarters and placed in a 20 oz extraction jar. The jar was placed on a rotary shaker at 300 rpm and extracted 2X for 20 min with 50 ml hexane. The hexane was decanted into a boiling flask and taken to dryness on a rotary evaporator at 40°C, then transferred 3X in 10 ml of isooctane for gas-liquid chromatography. Based on a GLC sensitivity limit of 25 pg and a 5 μl injection volume, the detection limit of this method is 0.01 ppb. Quantities below 0.01 ppb were reported as a trace. Recovery studies were conducted in water at the 20 ppb level by fortifying four 500 ml water samples from each pond with 10 μg of phenthoate, and with the α-cellulose pads at the 0.1 and 1.0 ng/cm^2 levels by fortifying three 4" x 4" α-cellulose pads/concentration with 5 ng and 50 ng, respectively. Recoveries, using the above extraction procedures, were 85.7 $\pm$ 9.1% for water, and 68.8 $\pm$ 3.3% and 82.2 $\pm$ 2.6% (0.1 ng/cm^2 and 1.0 ng/cm^2, respectively) for pads. The apparatus used to assay the phenthoate was a Tracor Model 550 gas chromatograph equipped with a flame photometric detector (FPD), 1.8 m x 2 mm ID glass column packed with 4% SP 2100 and 2% SP 2401 on 100 to 120 mesh Supelcoport. Conditions were: oven temperature 195°C, detector temperature 300°C, and an injector port temperature of 200°C. The nitrogen flow rate was 40 ml/min; hydrogen, 60 ml/min; and air flow of 100 ml/min.

RESULTS AND DISCUSSION

Chemical characteristics of the two ponds are given in Table 1; water temperatures and dissolved oxygen levels are presented in Table 2.

Each sampling day, the shallows (near shoreline) of the control and treatment ponds were inspected for dead or dying aquatic organisms. In the control pond, no dead or dying aquatic organisms were observed. Uncaged fish in the control pond were still active postspray and would retrieve Purina Catfish Chow pellets from the surface of the water along the shoreline. Fish in the control pond had no observable problems and a healthy appearance. In the treatment pond, no dead or dying aquatic organisms were observed 3 h postapplication. On day 1 postapplication dragonflies (_Erythrodiplax minuscula_), cove-headed grasshoppers (_Neoconocephalus_ sp.), angular-winged katydid (_Microcentrum rhombifolium_), and various species of freshwater gastropods (snails) were observed dead or dying on the surface of the pond. One dead bass and approximately 25 to 30 bluegills (1" to 2") were also retrieved floating at the grassy edge of the lake. On day 2 postapplication, more dead fish (3 bass (4" to 6"), 10 to 15 small bluegills (1" to 2"), 3 medium bluegills (2" to 3"), and one large bluegill (8")) were retrieved. During the next several days, additional dead fish appeared on the surface of the treatment pond. No live fish

Table 1. Chemical Characteristics of the pond water

Days postspray	-1 Treatment	-1 Control	9 Treatment	9 Control
Total hardness (ppm)	70	21	80	24
Ca hardness (ppm)	41	12	44	12
Mg (ppm)	29	9	36	12
Phenolphthalein alk. (ppm)	0	0	0	0
Total alkalinity (ppm)	50	11	40	18
Chloride (ppm)	30	28	32	22
Phosphate (ppm)	B.D.	B.D.	B.D.	B.D.
Specific conductance (MM)	192	93	220	110
pH	6.9	6.8	6.1	6.3
Fe (ppm)	0.07	0.20	0.05	0.04
SiO_2 (ppm)	0.55	6.60	0.50	4.98
SO_4 (ppm)	24.9	10.0	12.8	9.8
Turbidity (J.T.U.)	1.25	12.00	0.60	1.00
Tss (ppm)	1	1292	156	147
Oil	neg.	neg.	neg.	neg.
24 h plate ct. (col/ml)	269	953	neg.	neg.

Table 1. Chemical characteristics of the pond water (contd).

Days postspray	14 Treatment	14 Control	25 Treatment	25 Control
Total hardness (ppm)	72	31	76	40
Ca hardness (ppm)	47	22	40	12
Mg (ppm)	25	9	36	28
Phenolphthalein alk. (ppm)	0	0	0	0
Total alkalinity (ppm)	42	12	48	16
Chloride (ppm)	32	27	32	24
Phosphate (ppm)	B.D.	B.D.	B.D.	B.D.
Specific conductance (MM)	188	96	190	95
pH	7.5	6.3	7.4	7.0
Fe (ppm)	0.07	0.23	0.03	0.18
SiO_2 (ppm)	1.10	6.60	1.40	8.30
SO_4 (ppm)	24.3	7.5	25.0	7.0
Turbidity (J.T.U.)	0.95	2.50	0.45	1.80
Tss (ppm)	80	100	11	31
Oil	neg.	neg.	neg.	neg.
24 h plate ct. (col/ml)	B.D.	24	19	53

Table 2. Water temperature and dissolved oxygen in the pond water

	Control pond		Treatment pond	
Days postspray	Water temp. (°C)	Dissolved oxygen (mg/L)	Water temp. (°C)	Dissolved oxygen (mg/L)
-0.04	30.5	9.6	N/A	N/A
1	32.9	9.1	33.2	8.4
2	32.5	8.2	29.8	8.5
3	24.5	7.5	25.5	8.4
4	25.0	7.7	32.5	8.7
5	24.0	7.2	27.0	7.6
6	24.0	7.1	25.0	7.8
7	23.5	6.5	24.5	7.2
8	23.5	6.0	24.5	8.7
Mean $\pm$ S.E.M.	26.7 $\pm$ 1.3	7.7 $\pm$ 0.4	27.8 $\pm$ 1.3	8.2 $\pm$ 0.2

were observed in the treatment pond from day 2 to day 8, but 3 small live minnows were observed at the boat launch on day 8. The fish in the treatment cages, when alive, appeared to be in shock and responded sluggishly when prodded.

No phenthoate was detected on the drift pads of the control pond. Table 3 presents the treatment pond drift pad data. The outlying sequence of pads generally received more drift than those at the pond edge. The western side of the treatment pond (inner pad nos. 1-19, outer pad nos. 1-32) received more drift than the eastern half (inner pad nos. 20-41, outer pad nos. 33-50), consistent with westerly winds observed during the application. Cage pads received an average of 10.0 $\pm$ 2.7 $\mu g/cm^2$.

The control pond water contained no measurable phenthoate on days -1, 0.125, 1 and 2 postspray. Control pond water samples for phenthoate analyses were not taken after day 2. Table 4 presents the treatment pond phenthoate water concentrations, together with statistical fits to two possible mathematical representations of the observed phenthoate concentration decline with time. The first is the customary first-order, exponential representation. A half-life of about 2 days results. But only 80% of the variation in phenthoate concentration with time is explained thereby, while fully 99% is explained with an inverse power fit. These phenthoate concentrations taken from the surface water of the treatment pond could have declined for a number of reasons, such as downward diffusion of phenthoate, sorption by aquatic plant and animal organisms or various chemical reactions. They are, however, the phenthoate levels to which the caged fish were exposed.

There are several interesting calculations possible from distance measurements taken at the treatment pond, together with the data presented in Tables 3 and 4. If the area from the top of the sloped bank to the treatment pond's edge is deleted, the actual pond is approximately elliptical in shape with major axis 48.8 m and minor axis 26.8 m. The surface area of such a pond is 1027 m^2. Using the average phenthoate concentration for the outer pads (Table 3), the pond received 4.45 $\mu g/cm^2 \times 1027\ m^2 = 45.7$ g total. Using the inner pads, this calculation is: 3.02 $\mu g/cm^2 \times 1027\ m^2 = 31.0$ g total. For the cage pads: 10.0 $\mu g/cm^2 \times 1027\ m^2 = 102.7$ g. These 3 values, having a mean of 60 $\pm$ 22 g, represent how much phenthoate contamination the treatment pond received on the basis of drift pad data alone. The estimated volume of the pond is 2667 m^3, corresponding to an average depth of 8.5 ft. If it is assumed that the pesticide was uniformly mixed throughout the pond's volume, concentrations of phenthoate within the pond, based on the average over the 3 types of pads, can be estimated: $(60 \pm 22\ g) \div 2667\ m^3 = .023 \pm .008\ g/m^3 = 23 \pm 8$ ppb.

Table 3. Phenthoate concentration ($\mu g/cm^2$) on treatment pond drift pads

Stake number	Inner sequence	Outer sequence	Stake number	Inner sequence	Outer sequence
1	1.77	3.51	26	0.60	7.58
2	--	3.32	27	0.95	2.11
3	2.40	3.00	28	0.98	1.56
4	3.11	3.74	29	0.69	3.06
5	6.23	5.13	30	0.64	2.17
6	5.16	0.66	31	0.39	2.29
7	6.68	8.36	32	0.43	1.46
8	6.79	7.83	33	0.59	1.86
9	3.59	3.56	34	--	1.16
10	6.85	11.02	35	0.80	0.93
11	7.80	6.81	36	1.15	1.24
12	7.24	8.80	37	2.30	0.76
13	5.45	7.86	38	0.84	0.97
14	3.53	12.40	39	0.83	1.03
15	3.01	8.44	40	1.61	0.62
16	--	5.75	41	1.69	1.57
17	7.06	4.29	42	--	1.49
18	3.92	5.69	43	--	1.54
19	--	12.67	44	--	2.37
20	5.30	15.12	45	--	2.99
21	3.34	7.32	46	--	2.30
22	3.48	6.46	47	--	1.34
23	2.24	4.20	48	--	0.95
24	0.95	10.04	49	--	1.88
25	1.17	8.94	50	--	2.24
			Avg.	3.02	4.45

This concentration, expected on the basis of pad observations, is somewhat lower than the 140.0 ± 27 ppb phenthoate measured in the water 3 h after application (Table 4). It is, however, not significantly different from the 21.8 ppb value for 24 h (Table 4). Thorough mixing of phenthoate throughout the pond had probably not yet been completed in only 3 h.

Table 4. Phenthoate concentration (ppb) in treatment pond water samples

Days postspray	Cage #1		Cage #2		Cage #3		Mean conc. ± SEM[a]
	In	Out	In	Out	In	Out	
-1	N.D.[b]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
0.125	168.6	93.4	52.8	149.3	129.5	246.3	140.0 ± 27.1
1	19.6	20.0	18.8	20.6	21.9	29.6	21.8 ± 1.6
2	16.5	16.5	5.7	13.5	12.7	7.3	12.0 ± 1.9
3	5.5	10.4	11.3	5.8	7.0	6.2	7.7 ± 1.0
4	7.7	5.8	6.9	6.0	5.8	6.7	6.5 ± 0.3
5	7.2	5.8	6.1	6.0[c]	6.9	5.1	6.2 ± 0.3
6	4.2	4.2	--	5.6[c]	--	--	4.6 ± 0.5
7	4.7	2.8	--	3.6[c]	--	4.3[d]	3.8 ± 0.4
8	2.3	2.5	--	2.7[c]	--	3.0[d]	2.6 ± 0.1
9	2.0	2.5	--	2.5[c]	--	1.9[d]	2.2 ± 0.2

Least squares fit of mean conc. to $C = C_0 \exp(-kt)$: C_0 = 41.0 ppb; k = 0.36 day^{-1} ($T_{1/2}$ = 1.9 day); R^2 = 0.80.
Least squares fit of mean conc. to $C = C_1/t^n$: C_1 = 21.8 ppb; n = 0.93; R^2 = 0.99.
[a]Standard error of the mean; [b]Non-detectable amount; [c]Sample taken at center of pond; [d]Sample taken at west side of pond.

The concentration of phenthoate in the spray tank was 20 lb AI/500 gal or 4.8 g/L. Using the estimate of 60 ± 22 g phenthoate received by the treatment pond, the corresponding amount of spray material is (60 ± 22 g) ÷ 4.8 g/L = 12 ± 5 L. About 12 L of spray material does not seem unreasonable for drift, inasmuch as approximately 64,000 L of the spray material was applied to the surrounding grove.

On the other hand, the formulation was 4E or 4 lb AI/gal or 0.482 g AI/ml. Although the spray crew has a record of being careful not to spill formulation when filling the tank, a spillage of 200 ml which could have drained into the lake is not impossible. Such a 200 ml spillage would contain 96 g phenthoate and lead to a 36 ppb concentration in the 2667 m^3 lake, even when thoroughly mixed throughout the lake volume. But this scenario is not a likely one, in our opinion.

The result of the phenthoate drift was death to the caged fish in the treatment pond (Table 5). To determine whether fish died preferentially in certain cages of the treatment pond, postspray,

Table 5. Fish mortality in the ponds

Days	Live fish in control pond			Live fish in treatment pond		
	1[b]	2	3	1	2	3
Prespray						
-4[a]	68	67	67	70	68	64
-1	66	52	57	63	38	49
-0.125	62	50	55	62	38	48
Postspray						
1	60	50	55	55	36	46
2	58	50	52	25	22	26
3	57	50	51	1	1	1
4	56	49	51	1	0	0

[a]Fish introduced to cages.
[b]Cage number.

Table 6. Chi-square contingency table for fish mortality, postspray

	Number dead after 4-day postspray period	Number alive after 4-day postspray period
Treatment pond	147	1
Control pond	11	156

Result: $X^2 = 266.5$, df = 1.

Table 7. Chi-square contingency table for fish mortality, prespray

	Number dead after 3-day prespray period	Number alive after 3-day prespray period
Treatment pond	54	148
Control pond	35	167

Result: $X^2 = 4.7$, df = 1.

an F-test for row-effect was made. Since no significant difference in death rate among the cages was found (F = 2.75, df = 2.4), the cages were combined in Table 6. The chi-square test in Table 6 shows a wide statistical difference between the two ponds in number of fish dying. The only question, in our opinion, which could be asked is: were the two ponds equivalent in fish mortality, prespray? The statistics in Table 7 do indicate that significantly more fish died in the treatment pond vs. the control pond prior to spraying. However, treatment with phenthoate widened this prespray difference greatly (X^2 increased from 4.7 to 266.5). Our conclusion is that under the conditions described, phenthoate would cause excessive fish mortality in and around Florida citrus spray operations. It is also likely that phenthoate causes significant mortality in gastropods and aquatic insects.

Acknowledgments. Supported by a commercial grant from Montedison USA, Inc. Technical assistance was provided by T. W. Bailey, R. M. Williams, and S. E. Polinski. Florida Agricultural Experiment Stations Journal Series No. 4850.

REFERENCES

US Dept of Interior (1980) in Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. [Johnson W & Finley M, ed]. Fish and Wildlife Service Resource Publ No. 37 98 pp

Received July 20, 1983; Accepted September 20, 1983