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DTA EVALUATION RECORD

CHEMICAL: Diuron, Bromacil, Alachlor, Dinoseb, Propanil.

FORMULATION: Technical % A.I. indicated in report.

CITATION: Acute and early life stage toxicities, bioconcentration, and metabolism of five herbicides in freshwater fish, by Daniel Call, Larry Brooke and Raymond Kent. Final draft. U.S. EPA Grant-No. R-806196-01, ERL-Duluth, ORD, EPA, Duluth, MN. (19

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Aquatic Biologist
HED/EEB

DATE REVIEWED: May 17, 1982

TEST TYPE: Acute and early life stage toxicity studies, uptake, biocentration and elimination. Fathead minnow (Pimephales promelas)

REPORTED RESULTS:

See charts

REVIEWER'S CONCLUSIONS: The study is scientifically sound but does not fulfill our guideline requirements for a flow-through acute study, embryo-larvae study and bioconcentration study.

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Materials and Methods

Test Procedures

Acute Toxicity Test

Tests were run in 17.5L glass aquaria containing 6 L of water. A proportional diluter system based on Mount and Brungs was used. The diluters delivered 0.5L to each tank 4 to 8 times an hour. There were 5 concentrations of the technical herbicide and a control. Each concentration and the control was run in duplicate; there were 20 30-day old fathead minnows in each chamber. Tests were run at 25°C. The water used was from Lake Superior and was neither filtered nor sterilized.

Early Life Stage Study

Early life stage studies were conducted with the same basic diluter and exposure chamber apparatus as in the acute test. The system was modified to permit incubation of eggs in oscillating 130 ml specimen bottles with 200 u mesh nylon screen bottoms. The bottles were oscillated at a rate of 6 times a minute. The sizes of the exposure chambers were reduced to confine the fish to a smaller volume of 4.5L.

Fifty fathead minnow eggs < 24 hr old were placed into the jars; there were 2 jars per treatment replicate. Dead eggs were removed daily, and upon completion of hatch the survivors were counted and 15 fry from each jar were released into the chamber, with 30 fry per chamber. Feeding was begun the day after hatching through the end of the test.

Bioconcentration Test

Bioconcentration tests were run with a modified diluter system using fluid metering pumps to deliver ^{14}C-labeled diuron to each treatment chamber. One liter of water (25°C) was delivered 4 times an hour to each treatment control chamber where it was mixed with the test compound before going into the test chamber. The test solution was a mixture of technical grade and radiolabeled compound dissolved in methanol. All chambers, including the control, received 100 mg/l methanol.

One hundred 30-day old fathead minnows were exposed to each concentration. Five fish were removed and individually analyzed on days 1,2,4,7,10,14,17 etc. until an equilibrium was achieved in total ^{14}C between the exposure water and the fish tissue. When a plateau concentration was achieved in the fish, the remaining fish were transferred to clean water for depuration; the flushing rate was 4L/hr. Depuration rates were determined by individually analyzing the samples of 5 fish on days 1,2,4,7,10, 14,17, etc. until ^{14}C activity had been nearly eliminated.

Measurements of Herbicide Concentrations in Water and Fish

Herbicide concentrations were measured analytically in the acute, early-life stage and bioconcentration studies.

The test chemicals were: 92.6% alachlor, 95% bromacil, 98% dinoseb, 98.6% diuron and 85.9% propanil.

Metabolite Characterization

Rainbow Trout In Vivo Experiments

Rainbow trout weighing 100 to 150 g were injected with 1 u Ci of ^{14}C-herbicide and kept at 10°C. After 24 hr blood, bile and liver samples were taken. These tissues and the aquarium water were analyzed for metabolites.

Following these experiments trout weighing 100-150 g were exposed for 4-5 days at 10°C to unlabeled herbicide dissolved in 10-12L of Lake Superior water at concentrations less than the acute lethal levels. The water was changed after the first 24 hr. to remove fecal material. The fish were not fed during the experiment. One day prior to sacrifice, individual fish were injected with 1.0 u Ci of ^{14}C-herbicide. The gall bladder and liver were analyzed for metabolites.

Results and Discussion

Water quality analyses during the experiments indicated that Lake Superior water is soft quality. The pH values ranged from 7.2 to 7.4 and D.O. was 90% to 98% of saturation in the acute studies and 73% to 90% in the early life stage studies.

Acute Studies

TABLE 1 LC_{50} Values and 95% Confidence Intervals for Acute Toxicity Tests with Replicates Combined. All Values Expressed in mg L^{-1}.

Compound	24 Hr LC_{50}	48 Hr LC_{50}	96 Hr LC_{50}	192 Hr LC_{50}
Alachlor	8.7 (8.1-9.4)	5.7 (5.4-6.1)	4.3 (3.9-4.8)	2.6 (2.3-3.0)
Bromacil	185 (179-191)	183 (177-189)	182 (177-188)	167 (162-174)*
Dinoseb	0.8 (0.7-0.8)	0.7 (0.6-0.7)	0.6 (0.6-0.7)	0.4 (0.4-0.5)
Diuron	23.3 (21.0-25.9)	19.9 (19.5-20.4)	14.2 (13.4-15.0)	7.7 (6.0-9.9)
Propanil	11.5 (10.8-12.2)	10.2 (9.6-10.8)	8.6 (7.7-9.5)	3.4 (2.4-4.8)

* Values are for 168 hr LC_{50}.

Fathead Minnow

The mean test concentration ranges of the herbicides in the acute tests were: alachlor (1.46 to 13.3 mg/l); diuron (5.54 to 24.20 mg/l); propanil (1.9 to 15.7 mg/l); and bromacil (1.08 to 20.7 mg/l).

Metabolite Characterization

Rainbow trout injected with ^{14}C-labeled herbicides readily metabolized all five compounds. From 75 to 98% of the injected doses were excreted within 24 hr. Metabolites of the herbicides accumulated in the bile in varing degrees from 1% (bromacil) to 22% (propanil) of the injected doses.

Herbicide Stabilty in Solution

Solutions of biomacil, dinoseb, diuron and propanil at concentrations approximating the 96-hr LC_{50} values for fathead minnow were made with Lake Superior water. One-liter stoppered bottles in duplicate were exposed to a 12L: 12D photoperiod at 25°C for 40 days (propanil was held at 20°C for 194 days).

Dinoseb declined by 21% and bromacil and diuron remained stable for 40 days. Propanil declined curvilinearly during the 194 day period with a 65-day half-life.

Reviewer's Evaluation

Materials and Methods

Test Procedures

The test procedures are acceptable, except the percents active ingredients are not indicated.

Statistical Analyses

No information is given regarding the methods used to calculate the LC_{50} values or used to determine statistical significance.

Results

Without the raw data for acute toxcity and early-life stage studies. The results cannot be verified.

Conclusions

1. Category: Supplemental
2. Rationale: Lack of raw data and information regarding statistical analyses used, and percents of the active ingredients.
3. Repairability: The study can be upgraded if the missing data and information are supplied and determined to be acceptable.

TABLE 2. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO ALACHLOR FOR 60 DAYS.

Parameter	Mean Alachlor Concentration (mg/l) 0.00	0.06	0.14	0.26	0.52	1.10
Mean percent hatch*	72.8	73.3	68.3	76.2	76.4	73.4
Mean percent abnormal and dead+	2.6	4.0	3.7	2.0	0.6	0.0
Mean number of survivors at 60 days‡	24.5	26.5	28.0	28.0	29.5	25.0
Mean wet weight at 60 days (g)	0.482	0.432	0.419**	0.403	0.423	0.316#
Mean total length at 60 days (mm)	37.1	36.2	35.6**	35.0	35.9	31.9#

* Live fry/total eggs.

\+ Abnormal (deformed) + dead fry/total fry at time of transfer.

‡ Based on 30 fry transferred to duplicate exposure chambers.

**Significantly different from controls ($_p$<0.05).

\# Significantly different from controls ($_p$<0.01).

TABLE 3. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO BROMACIL FOR 60 DAYS.

Parameter	Mean Bromacil Concentration ($mg \cdot l^{-1}$) 0.0	1.0	1.9	4.3	12.0	29.0
Mean percent hatch*	66.0	68.8	58.4	58.5	60.4	72.3
Mean percent abnormal and dead+	5.9	6.9	7.4	8.3	8.4	11.5
Mean number of survivors at 60 days‡	26.5	28.5	29.5	29.5	27.5	26.5
Mean wet weight at 60 days (g)	0.479	0.410#	0.420**	0.381#	0.374#	0.326#
Mean standard length at 60 days (mm)	29.8	28.4**	28.7	27.9#	27.7#	26.4#

* Live fry/total eggs.

\+ Abnormal (deformed) + dead fry/total fry at time of transfer.

‡ Based on 30 fry transferred to duplicate exposure chambers.

**Significantly different from controls ($p < 0.05$).

\# Significantly different from controls ($p < 0.01$).

TABLE 4. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) EXPOSED TO DINOSEB FOR 60 DAYS.

Parameter	Mean Dinoseb Concentration (ug/l) 0.0	0.4	1.7	4.3	14.5	48.5
Mean percent hatch*	74.9	85.0	86.3	80.0	73.5	79.7
Mean percent abnormal and dead+	4.6	0.6	3.4	3.5	6.0	1.5
Mean number of survivors at 60 days‡	26.5	23.5	24.5	26.0	16.0	2.5#
Mean wet weight at 60 days (g)	0.599	0.683	0.733***	0.647	0.679	0.517#
Mean total length at 60 days (mm)	39.7	41.3	42.2**	39.5	40.5	35.2

* Live fry/total eggs.

+ Abnormal (deformed) + dead fry/total fry at time of transfer.

‡ Based on 30 fry transferred to duplicate exposure chambers.

Significantly different from controls ($p<0.01$).

***Significantly heavier than controls ($p<0.01$ two-tailed Dunnett's test).

** Significantly longer than controls ($p<0.05$ two-tailed Dunnett's test).

TABLE 5. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO DIURON FOR 60 DAYS.

Parameter	Mean Diuron Concentration (ug/l) 0.0	2.0	5.0	11.5	26.4	61.8
Mean percent hatch*	67.9	77.9	75.0	71.8	67.9	66.1
Mean percent abnormal and dead+	2.2	0.6	1.3	3.7	8.2	15.0#
Mean number of survivors at 60 days‡	24.5	26.5	28.0	21.5	22.5	7.5**
Mean wet weight at 60 days (g)	0.568	0.568	0.563	0.619	0.563	0.496
Mean total length at 60 days (mm)	32.2	32.3	32.4	32.3	31.0	29.1

* Live fry/total eggs.

+ Abnormal (deformed) + dead fry/total fry at time of transfer.

‡ Based on 30 fry transferred to duplicate exposure chambers.

** Significantly different from controls ($p<0.05$).

\# Significantly different from controls ($p<0.01$).

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TABLE 6. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO PROPANIL FOR 54 DAYS.

Parameter	Mean Propanil Concentration (ug/l) 0.0	0.4	0.6	1.2	2.4	3.8
Mean percent hatch*	75.9	80.5	70.2	63.4	64.0	56.6
Mean percent abnormal and dead+	3.0	5.5	10.4	9.4	13.5	65.8
Mean percent fry survivors at 54 days‡	93.4	72.5	50.0	16.6#	0#	0#
Mean wet weight at 60 days (g)	0.590	0.558	0.491	0.448	--	--
Mean dry weight at 60 days	0.152	0.132	0.119**	0.113	--	--
Mean total length at 60 days (mm)	38.3	36.7	34.2#	33.1#	--	--

* Live fry/total eggs.

\+ Abnormal (deformed) + dead fry/total fry at time of transfer.

‡ Based on mortality of 30 fish/chamber through day 30 post-hatch and 20 fish/chamber between days 30-54.

**Significantly different from controls (ρ_p<0.05).

Significantly different from controls (ρ_p<0.01).

TABLE 7. BIOCONCENTRATION, METABOLISM AND ELIMINATION OF FIVE TEST HERBICIDES IN FATHEAD MINNOWS (Pimephales promelas) EXPOSED TO VARIOUS WATER CONCENTRATIONS.

Herbicide	Mean H_2O concentration (µg/l),	^{14}C Bio-concentration factor	Percent parent compound at end of uptake*	Percent depuration in 24 hr.	Percent depuration at end of test (days of depuration)
Alachlor	0.66	50.2	13.2	84.0	96.4 (14)
	9.95	41.4		78.3	99.8 (14)
Bromacil	4.25	2.8	nd+	nd	nd
	35.11	3.5		nd	nd
Dinoseb	0.62	61.5	2.3	67.4	94.6 (14)
	7.22	64.1		73.8	97.9 (14)
Diuron	3.15	157.0	1.3	83.8	98.7 (21)
	30.40	143.7		76.4	99.0 (21)
Propanil	0.34	69.0	1.8	80.0	96.4 (10)
	5.09	111.3		80.8	95.4 (10)

* Determined as portion of ether-extractable fraction that chromatographed by TLC as parent compound from fish exposed to higher concentrations of test herbicides.

+ Not determined.