028201

10-18-79

VALIDATION SHEET

FORMULATION:	CHEMICAL NAME	Validator:	Date
% a.i.	3,4-Dichloropropionanilide	J. S. Leitzke	10/18/79
86% (Adler, Rohm & Haas, 10/4/79)		Test Type: Fish Acute LC_{50} - Fathead minnow; 60-day Embryo-Larvae - Fathead minnow; Residue Uptake - Fathead minnow; Metabolism - Rainbow trout; Herbicide Stability in Solution	

CITATION: Accession No. [not assigned yet]; D. J. Call, R. Kent and L.T. Brook, 1979, Estimates of "no effect" concentrations of selected pesticides in freshwater organisms, Univ. Wisc.-Superior, 1st, 2nd & 3rd Quarterly Progress Report to EPA, 1978-1979; In: EEB Propanil Registration Review file. (plus personal communications from D. Call, 10/15/79 & 10/29/79)

VALIDATION CATEGORY:

1) Fish Acute LC_{50} - Supplemental
2) 60-day Embryo-Larvae - Core
3) Residue Uptake - Core
4) Metabolism - Core
5) Herbicide Stability in Solution - Core

RESULTS:

1) Fish Acute Flow-Through LC_{50} - Fathead minnow (Pimephales promelas) - in ppm AI

24-hr.	48-hr.	96-hr	192-hr.
11.6	10.2	8.6	3.2

2) 60-day Embryo-Larvae - Fathead minnow (Pimephales promelas) egg-to-Fingerling Maximum Acceptable Toxicant Concentration (MATC) = 0.4-0.6 ppb AI
Application Factor (96-hr LC_{50}/MATC) = 22,000

3) Residue Uptake - Fathead minnow (Pimephales promelas)

Bioconcentration Factors at 0.5,5.0,5.0 and 50 ppb (nominal) = 69.0, 111.3, 114.4 and 66.3, respectively
Percent Loss at End of Withdrawal = ca. 96%

4) Metabolism-Rainbow trout (Salmo gairdneri)

Principal Metabolites are 1) either 3',4',-dichloro-2-hydroxypropionanilide or 3',4'-dichloro-3-hydroxypropionanilide and 2) 3,4-dichloroaniline (DCA). Remaining in Bile 24-hr after injection with ^{14}C- labeled propanil was 9-22% of original activity.

Found in Water after 24-hr was 75%.

5) Herbicide Stability in Solution

Half-life in Lake Superior water = 65 days

DISCUSSION & VALIDATION RATIONALE:

1) Fish Acute Flow-Through LC_{50}

Two Replicate Tests were run generally according to standard prodedures for flow-through tests, except that the fathead minnow (generally not an accepted test species for acute tests) was used. Some of the test conditions were as follows: temperature 25.0°C (24.1-26.6°C), DO (as % saturation) 90.0 (± 1.4), total hardness (as ppm $CaCO_3$) 51.3 (± 6.7), pH 7.5 (± 0). LC_{50}'s were calculated in terms of actual concentrations in water (and not nominal concentrations) using the trimmed Spearman-Karber method. Replicate and mean LC_{50} values (in ppm) are tabulated below:

	24-hr.	48-hr.	96-hr.	192-hr.
	11.9 (9.9-14.3)	10.0 (8.9-11.3)	8.6 (7.4-9.9)	3.9 (2.8-5.6)
	11.4 (9.6-13.5)	10.5 (9.6-11.5)	8.5 (7.0-10.2)	2.6 (1.7-4.2)
Mean	11.6	10.2	8.6	3.2

Note that toxicity increased at a faster rate as the test proceded.

2) 60-day Embryo-Larvae

A standard proportional-diluter provided continuous exposure to ≤24-hr-old fertilized fathead minnow eggs and subsequently hatching fry surviving fingerlings for 54 days at 5 actual duplicated concentrations (and not nominal concentrations) of 0.0, 0.4, 0.6, 1.2, 2.4 and 3.8 ppb. (In the first attempt more than 50% of hatched fry from exposed eggs died within 48-hr at the lowest concentration 5.6 ppb. On the other hand, newly hatched fry from unexposed eggs had no mortalities at 5 ppb [personal communication, D. Call, 10/15/79; letter attached to Quarterly Repts.]). Eggs were placed in

incubation jars (approx. 50 eggs per jar, 2 jars per replication), and after hatching a total of 30 fry were released into the chamber (later cut back 20; D. Call, pers. comm.)

Some of the test conditions were as follows: temperature 25.3°C (23.6-27.2°C), DO (as % saturation) 73.7 (± 28.9), total hardness (as ppm $CaCO_3$) 57.9 (± 2.1), pH 7.3 (± 0.3). Results were statistically analyzed by one-way analysis of variance with a one-tailed Dunnett's test. Results are tabulated below:

Need raw data to re evaluate this study

Parameter	Mean Propanil Concentration (ug/1) CONTROLS 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 survival at 54 days***	93.4	72.5	50.0	16.6##	0##	0##
Mean wet weight at 54 days (g)	0.590	0.558	0.491	0.448	---	---
Mean dry weight at 54 days (g)	0.152	0.132	0.119#	0.113	---	---
Mean total length at 54 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 maximum per chamber through day 30 post-hatch and 20 fish maximum per chamber between days 30 and 54 post-hatch.
\# Significantly different from controls ($p<0.05$).
\## Significantly different from controls ($p<0.01$).

The most common symptoms of poisoning were swelling of yolk-sac around the heart and (pericardial edema) and bloated abdomens streaked with red (pers. comm, D. Call, 10/15/79). The resulting Application Factor (96-hr LC_{50} divided by MATC) is (8.6 ppm/0.4 ppb =) ca. 22,000.

3) Residue Update

Two sets of residue uptake tests were run, at continuous, nominal concentrations 5.0 & 50 ppb and 0.5 & 5.0 ppb ^{14}C-labelled propanil using modified proportional diluters. One-hundred 30-day-old fathead minnows per chamber were used. Two tests were necessary because 14.5 and 16.4% mortality occurred in the 5.0 and 50 ppb nominal concentrations (3.96 and 53.49 ppb actual) chambers over the 21-day exposure period. Depuration in the first run was for 21 days. The second run at 0.5 and 5.0 ppb nominal concentration (0.34 and 5.09 ppb actual) had 17 days exposure and 10 day depuration. The results are tabulated below:

Mean H_2O concentration (ug/l)	Bio-concentration factor	Percent parent compound at end of uptake*	Percent depuration in 24 hr.	Percent depuration at end of test (days of depuration
3.96**	114.4		80.1	95.6 (21)
53.49**	66.3	1.8	76.2	95.9 (21)
0.34***	69.0		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.

** First propanil run.

*** Second propanil run.

In all tests both initial uptake and elimination of ^{14}C were rapid.

4) Metabolism

One rainbow trout (100-150 gm) in 10°C water, was injected with ^{14}C- labelled propanil and sacrificed 24-hr. later for analysis of bile and liver. Metabolites were analyzed first by thin layer Chromatography (TLC) on silica gel coated glass plates followed by mass spectral analysis.

TCAB (3,3',4,4'-tetrachloroazobenzene) was found in the mass spectral analysis but not in the TLC and therefore the authors concluded that 3,4-dichloroaniline (DCA) photochemically converted to TCAB prior to mass spectral analysis.

5) Herbicide Stability in Solution

Propanil stability in Lake Superior water was determined over 194 days at 20°C. Duplicate 500 ml stock solutions had initial concentrations of approximately the 96-hr. LC_{50} for fathead minnows. Propanil decreased curvilinearily with a half-life of 65 days. Principal degradate determined by mass spectral analysis was 3,4-dichloroaniline.

Appendix I

Final Results of Previously Submitted Data Recalculated, Corrected and Extrapolated As Necessary

Tag No.	Species	Test	Reported Results	Recal. Results (if nec.)	Material (% AI)	In terms of AI/Form	Corrected Results in AI	Extrapolated Results (if nec.)
20	Mallard (Anas platy-chnchos)	Mult. Dose Acute Oral LD_{50}	(Betz) 375 mg/kg/day	(Actual) 320 mg/kg/day	36.5%	Form.	116.8 mg/kg/day	
21	Japanese Quail (Coturnix Coturnix japonica)	Mult. Dose Acute Oral LD_{50}	160 mg/kg/day	—	36.5%	Form.	58.4 mg/kg/day	
22	Europ. Strl. (Sturnus vulgaris)	Mult. Dose Acute Oral LD_{50}	3000 mg/kg/day	—	36.5%	(Form.)	1095 mg/kg/day	
22	Engl. Sprr. (Passer domesticus)	Mult. Dose Acute Oral LD_{50}	187.5-375 mg/kg/day	—	36.5%	(Form.)	68.4 - 136.9 mg/kg/day	
23	Rainbow Trout (Salmo gaird-neri)	48-hr LC_{50}	4.0 ppm	—	Tech. (?)	(AI)	—	
24	Lake Emerald Shiner (Notropis atherinoides)	96-hr TLM	7.5 ppm AI	(other) 4-hr - 13.5 24-hr 7.5 48-hr 7.5	STAM F 34 (?)	AI	—	
25	Goldfish (Carassius auratus)	48-hr LC_{50}	22.4 ppm	24-hr 24.03 48-hr 21.27	36.5%	Form.	24-hr 8.77 48-hr 7.76	96-hr 6.87
25	Brown Bullhead (Ictalurus nebulosus)	48-hr LC_{50}	22.2 ppm	24-hr 23.39 48-hr 22.09	36.5%	Form.	24-hr 8.54 48-hr 8.06	96-hr 7.61

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Tag No.	Species	Test	Reported Results	Recal. Results (if nec)	Material (% AI)	In terms of AI/Form	Corrected Results in AI	Extrapolated Results (if nec)
26	Rainbow Trout (*Salmo gairdneri*)	48-hr LC_{50}	7.4 ppm	24-hr 9.65 48-hr 6.95	36.5%	Form.	24-hr 3.52 48-hr 2.54	96-hr 1.83
27	*Daphnia pulex*	48-hr LC_{50}	11.8 ppm	24-hr 14.11 48-hr 11.44	36.5%	Form.	24-hr 5.15 48-hr 4.18	
28	*Daphnia magna*	26-hr LC_{50}	4.8 ppm (3.8-6.6)	—	Tech. (?)	(AI)	—	

24-hr LC50
Goldfish

15.5
0.
10.

21.
2.
10.

28.
8.
10.

32.
10.
10.

15.701 M
-16.680 YINT
1.158 LW M
0.513 CHI²

24.032 LD50
21.951 LOCL
26.311 UPCL

19.913 LD10
17.237 LOCL
23.006 UPCL

29.003 LD90
25.762 LOCL
32.652 UPCL

48-hr LC50
Goldfish

10.
0.
10.

15.5
1.
10.

21.
4.
10.

28.
9.
10.

32.
10.
10.

11.032 M
-9.647 YINT
1.232 LW M
0.699 CHI²

21.268 LD50
19.014 LOCL
23.790 UPCL

16.275 LD10
13.550 LOCL
19.548 UPCL

27.793 LD90
23.812 LOCL
32.439 UPCL

24-hr LC50
Brown Bullhead

10.

10.
0.
10.

21.
2.
10.

24.
6.
10.

28.
9.
10.

37.
10.
10.

17.158 M
-18.489 YINT
1.144 LW M
0.046 CHI²

23.388 LD50
21.816 LOCL
25.074 UPCL

19.692 LD10
17.167 LOCL
22.587 UPCL

27.779 LD90

48-hr LC50
Brown Bullhead

10.
0.
10.

21.
3.
10.

24.
8.
10.

28.
10.
10.

25.131 M
-28.781 YINT
1.096 LW M
0.073 CHI²

22.091 LD50
20.885 LOCL
23.366 UPCL

19.643 LD10
17.608 LOCL
21.913 UPCL

24.845 LD90
22.749 LOCL
27.133 UPCL

24-hr LC50

Rainbow Trout

4.
0.
10.

6.3
2.
10.

8.
4.
10.

16.
8.
10.

25.
10.
10.

5.050 M
0.029 YINT
1.578 LW M
1.043 CHI²

9.647 LD50
7.758 LOCL
11.996 UPCL

5.377 LD10
3.939 LOCL
7.338 UPCL

17.308 LD90
12.067 LOCL
24.827 UPCL

48-hr LC50

Rainbow Trout

2.5
0.
10.

4.
2.
10.

6.3
3.
10.

8.
6.
10.

16.
10.
10.

5.192 M
0.630 YINT
1.558 LW M
1.882 CHI²

6.946 LD50
5.655 LOCL
8.532 UPCL

3.934 LD10
2.849 LOCL
5.432 UPCL

12.264 LD90
8.448 LOCL
17.805 UPCL

Daphnia pulex

24-hr LC50

10.
9.
30.

16.
17.
30.

25.
24.
30.

40.
30.
30.

4.145 M
0.236 YINT
1.743 LW M
1.709 CHI²

14.110 LD50
12.018 LOCL
16.566 UPCL

6.922 LD10
4.944 LOCL
9.690 UPCL

28.763 LD90
22.671 LOCL
36.493 UPCL

Daphnia pulex

48-hr LC50

10.
11.
30.

16.
24.
30.

25.
30.
30.

6.661 M
-2.050 YINT
1.413 LW M
0.654 CHI²

11.442 LD50
10.094 LOCL
12.971 UPCL

7.346 LD10
5.706 LOCL
9.457 UPCL

17.823 LD90
14.968 LOCL
21.223 UPCL