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EEE BRANCH REVIEW

DATE: IN 9/15/77 OUT 6/13/78 IN _____ OUT _____ IN _____ OUT _____

FISH & WILDLIFE ENVIRONMENTAL CHEMISTRY EFFICACY

FILE OR REG. NO. 241-243

PETITION OR EXP. PERMIT NO. _____

DATE DIV. RECEIVED September 8, 1977

DATE OF SUBMISSION _____

DATE SUBMISSION ACCEPTED _____

TYPE PRODUCTS(S): I, D, (H) F, N, R, S _____

DATA ACCESSION NO(S). 096342

PRODUCT MGR. NO. 25 (Taylor)

PRODUCT NAME(S) Prowl

COMPANY NAME American Cyanamid Company

SUBMISSION PURPOSE Final report of chronic fish study

CHEMICAL & FORMULATION N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine

100.5 Purpose of Submission

Submission of chronic fish study for Prowl Technical for final evaluation by Environmental Safety Section.

101.0 Chemical and Physical Properties

See previous Environmental Safety reviews.

102.0 Behavior in the Environment

See review by N. J. Cook (March 28, 1977).

103.0 Toxicological Properties

103.4 Chronic Toxicity

103.4.3 Fish:

DATA REVIEW NUMBER: (ES) X-1

TEST: Chronic Fish Study

CHEMICAL: Prowl Technical (98.3%)

TEST SPECIES: Fathead minnow

REGISTRANT: American Cyanamid Co. (Test conducted by Bionomics, Aquatic Toxicology Lab).

DATE OF TEST: September 8, 1977

ACCESSION NO.: 96342

EVALUATION CATEGORY: ~~Supplemental~~ upgraded to Core based on EEB statistical analysis

CATEGORY REPAIRABILITY: N. A.

RESULTS: (1) A flow-through acute toxicity test on fathead minnows resulted in: 6-day LC_{50} = 332 (272-404) ppb; 14-day LC_{50} = 137 (103-182) ppb (see Table 2 for dose-mortality data).

(2) 1st Generation (0-64 days): No significant differences in percent hatchability were observed among egg groups incubated in the control, solvent control, and concentration of CL-92,553 (Prowl Technical) as high as (level 1) 40 µg/ℓ (Table 6).

The percentage fry which survived 30 days continuous exposure to levels 1 and 2 were significantly lower than survival of fry in control, solvent control, and those exposed to levels 3, 4, and 5 (Table 6). Virtually all of the mortality of fry in these two concentrations occurred during the initial week of exposure. Total lengths of 30-day old fry were similar for controls and all concentrations of CL-92,553 to which fry were exposed. After 60 days exposure, fry survival was unchanged from that observed on day 30 and growth of fry (total length, wet weight) remained unaffected by any of the concentrations of CL-92,553 to which fry were exposed.

(3) 1st Generation (64-288 days): Between test-day 64, when first generation fish were released to the spawning chamber, and day 288 when exposure of these

fish was terminated, survival of fathead minnow in all treatments was excellent. A total of 8 mortalities occurred during this period and none of the dead fish had been exposed to the three highest treatment concentrations of CL-92,553. When exposure of adult fish was terminated (day 288), dissection of each fish revealed that the desired ratio of three males and seven females had not been achieved in all tanks (Table 7). This was due to the presence of later maturing males which had been identified as females from their external appearance at the start of the spawning period (day 168). Analysis of variance indicated that total lengths and weights of male and female fathead minnows exposed 288 days to concentrations of CL-92,553 as high as level 1 did not differ significantly from lengths and weights of fish in control and solvent control treatments.

(4) Spawning: Spawning began in most tanks about day 170 and continued through day 281. All spawning parameters (presented in Table 8) were similar for control fish and fish exposed to the highest treatment concentration (level 1) which averaged 43 µg/ℓ during the spawning period. The total number of eggs, eggs per spawn and eggs per female from fathead minnows exposed to level 3 were lower than those observed in all other treatments. This is not considered to be an effect of exposure to CL-92,553 in view of the higher egg numbers obtained from fish exposed to levels 1 and 2. (See Discussion for this reviewer's interpretation of these data). Fathead minnows in level 2 produced significantly higher numbers of eggs per spawn than did fish in control aquaria. The mean percentage hatchability of eggs spawned by fish exposed to levels 1 and 2 appear to be somewhat lower than percentage hatch in other treatments. Analysis of variance of these data indicated that the differences observed were not significant ($P > 0.05$).

(5) 2nd Generation (0-60 days): Survival of second generation (F_1) fry exposed 30 days to level 1 which had an average concentration of 47 µg/ℓ CL-92,533 was lower than survival of F_1 fry in control, solvent control and concentrations of CL-92,553 ≤21 µg/ℓ (levels 2-5) (Table 9). As in the first generation (F_0) fry exposures, most of the mortality of F_1 fry exposed to this concentration occurred during the first week

after hatching. Total length and wet weight of F_1 fry were not affected by 30 days exposure to mean measured concentrations of CL-92,553 as high as level 1.

After the completion of the initial series of F_1 fry exposures, additional fry groups were exposed to obtain information on the critical life stage (newly hatched fry). Solvent control eggs were incubated in the high treatment tanks and resulting fry did not survive as well after 30 days exposure to level 1 when compared with survival of fry in control and lower concentrations of CL-92,553 (levels 2-5) (Table 10). Eggs spawned by fathead minnows exposed in the high treatment were transferred to the solvent control and survival of resulting fry was excellent after 30 days. These data indicated that effects of CL-92,553 on fry survival were not cumulative from exposed parents to their progeny, but resulted from direct exposure of fry to a lethal concentration in water. Observations of additional F_1 fry groups exposed for 60 days in the control and CL-92,553 concentrations of level 2 to 5 indicated no significant differences in percentage survival, total length and wet weight after both 30 and 60 days.

(6) Residue analyses (fish tissue): Concentrations of CL-92,553 in whole fish tissue after 64 days exposure of first generation fathead minnows were directly proportional to aqueous concentrations to which these fish were exposed (Table 11). Bioconcentration factors (average concentration in tissue/average concentration in water) were close to 1200X for each of the five treatments of CL-92,553. After 168 days, residues of CL-92,553 detected in whole fish tissue had increased significantly, particularly for fish exposed to level 1 (2200 X). At the end of exposure of first generation fish (day 288), residues of CL-92,553 in tissues had decreased, approximating those observed for fish exposed 64 days. CL-92,553 is lypophylic and the temporary increase in tissue residues of CL-92,553, observed in fish exposed 168 days, could be related to the large amount of fatty tissue observed in the viscera of fish which were sampled at this time. Once fish had completed spawning (day 288) these stored fats were not observed in comparable quantities and tissue residues of CL-92,553 had decreased.

Residues of CL-92,553 in the carcass portion of fathead minnows exposed for 168 days were similar to residues observed previously for whole fish exposed 64 days (Table 13). Residues in the viscera of these same fish were substantially higher than in the carcass and accounted for the increase in calculated whole fish concentrations discussed previously. These data support the hypothesis that large quantities of fatty tissue in the viscera of fish exposed 168 days were responsible for the increased concentrations observed in whole fish at this time.

(7) Residue analyses (fish eggs and fry): Fathead minnow eggs contained lower residues of CL-92,553 than those observed in the tissues of fish exposed to the similar concentrations in water (Table 12). Bioconcentration factors for eggs were in the range of 200-500X and were lower than those calculated for first generation fish exposed 64 days (1200X). With the exception of fry exposed to level 1 for 30 days, second generation fathead minnows generally did not accumulate tissue residues comparable to those of first generation fish.

(8) Elimination of Residues: The bulk of the CL-92,553 which was present in both the carcass and viscera of fish exposed 168 days was eliminated within 5 days after transferring these fish to aquaria receiving uncontaminated well water (Table 13). The concentration of CL-92,553 in the viscera of fathead minnows exposed to level 1 for 168 days had decreased from 310 µg/g to 2.9 µg/g after 5 days of depuration. This represents an elimination of 99 percent of accumulated residues over a period of 5 days in uncontaminated well water.

After the completion of spawning, residues of CL-92,553 in both the carcass and viscera of fathead minnows exposed 288 days had decreased significantly from those observed in fish exposed 168 days (Table 14). The decrease was most pronounced in the viscera of fish exposed 288 days, again indicating that visceral fatty tissue stored prior to spawning (day 168) was responsible for a temporary increase in tissue concentrations of CL-92,553.

Fathead minnows exposed to level 1 for 288 days contained similar concentrations in both the carcass and viscera after 1 and 2 days of depuration. After the third day of depuration, approximately 75 percent of the CL-92,553 had been eliminated from both the carcass and viscera. This observation, when combined with depuration of fish exposed 168 days, indicates that the bulk of the CL-92,553 accumulated in fish tissue is eliminated between 3 and 5 days after fish are transferred to a flowing system of water uncontaminated by CL-92,553.

TABLE 2. OBSERVED PERCENTAGE MORTALITIES AT 48 HOUR INTERVALS DURING FLOW-THROUGH ACUTE EXPOSURE OF FATHEAD MINNOWS TO CL-92,553.

CL-92,553 concentrations (µg/ℓ)	Observed percentage mortalities (# dead/20 fish x 100)						
	days/2	4	6	8	10	12	14
490	35	85	100	100	100	100	100
370	0	0	30	75	90	100	100
280	0	5	15	95	100	100	100
210	0	0	0	15	50	90	90
160	0	0	0	10	15	20	25
120	0	0	5	20	30	35	35
90	5	10	15	15	15	15	15
control	0	0	0	0	0	0	0

TABLE 3. MEASURED WATER QUALITY PARAMETERS DURING CHRONIC EXPOSURE OF FATHEAD MINNOWS TO CL-92,553.

Parameter	Mean ± S.D.	Range	Number of samples
Hardness (mg/ℓ)	32.7 ± 6.8	23-41	21
Alkalinity (mg/ℓ)	39.0 ± 11.5	27-53	14
Acidity (mg/ℓ)	4.5 ± 0.5	3.8-4.8	7
Dissolved O_2 (mg/ℓ)	7.8 ± 0.8	5.4-8.3	502
pH	-	7.1-7.7	77

TABLE 6. EFFECTS OF FIRST GENERATION FATHEAD MINNOWS (Pimephales promleas) DURING CONTINUOUS EXPOSURE TO CL-92,553.

Exposure levels	0-3 days (eggs) Mean measured concentration (µg/ℓ)		Hatch (%)	0-30 days Mean measured concentration (µg/ℓ)		Survival (%)	Total length (mm)	0-60 days Mean measured concentration (µg/ℓ)		Survival (%)	Total length (mm)	Wet weight (g)
1	40 ± 4.4	A	76	36 ± 11	A	58	21 ± 3	36 ± 9.7	A	58	31 ± 4	0.29
		B	92		B	68	22 ± 3		B	68	31 ± 4	0.32
2	30 ± 4.3	A	97	22 ± 7.9	A	78	22 ± 4	21 ± 7.3	A	78	31 ± 3	0.23
		B	96		B	60	23 ± 3		B	60	34 ± 4	0.30
3	9.4 ± 0.87	A	97	7.8 ± 2.9	A	100	23 ± 2	7.7 ± 2.6	A	98	31 ± 3	0.24
		B	95		B	100	22 ± 2		B	98	30 ± 2	0.21
4	5.8 ± 1.5	A	99	4.7 ± 1.8	A	95	23 ± 3	4.8 ± 1.6	A	93	30 ± 3	0.21
		B	88		B	98	24 ± 2		B	95	31 ± 2	0.26
5	2.7 ± 0.98	A	98	2.5 ± 1.5	A	88	23 ± 2	2.4 ± 1.3	A	88	32 ± 4	0.27
		B	98		B	98	23 ± 2		B	98	30 ± 3	0.23
	solvent control	A	97	solvent control	A	98	24 ± 2	solvent control	A	98	29 ± 3	0.28
		B	80		B	100	23 ± 2		B	100	30 ± 3	0.25
	control	A	96	control	A	95	23 ± 2	control	A	95	33 ± 4	0.28
		B	97		B	98	23 ± 2		B	95	30 ± 3	0.21

TABLE 7. [illegible] SEXUAL MATURITY, AND GROWTH OF FATHEAD MINNOWS EXPOSED [illegible] TO DI-n-BUTYL PHTHALATE.

Exposure level	Mean measured concentration for 1-388 (µg/l)		Number of survivors			Total length (mm)		Dry weight (mg)	
			Mature males	Mature females	Immature	Males	Females	Males	Females
1	38	A	3	7	0	69(5)[a]	60(3)	3.63	2.17
		B	3	7	0	73(2)	55(3)	4.50	1.62
2	19	A	4	5	1	70(4)	59(1)	3.61	1.49
		B	3	7	0	70(6)	57(3)	3.99	1.97
3	8.0	A	4	4	2	76(5)	51(7)	5.03	1.21
		B	3	6	1	72(3)	55(3)	4.18	.85
4	5.1	A	4	5	1	75(6)	57(1)	4.67	1.54
		B	3	6	0	74(8)	55(4)	4.84	1.57
5	2.7	A	4	4	2	73(5)	59(4)	4.13	1.84
		B	4	3	2	70(6)	49(6)	2.76	1.07
	solvent control	A	3	5	1	73(2)	57(3)	4.69	1.69
		B	3	6	1	72(3)	54(4)	3.19	1.41
	control	A	3	6	0	76(2)	57(3)	5.52	1.48
		B	2	7	1	76(5)	57(5)	5.04	1.60

[a] Mean and standard deviation.

er	Eggs per female	Hatchability Mean ± S.D.	(N)[b]
	726	71 ± 25	12
	549	80 ± 14	14
	1,483	73 ± 30	13
	1,953	89 ± 9.4	19
	437	92 ± 7.7	10
	496	83 ± 14	10
	1,360	94 ± 4.8	16
	1,444	90 ± 7.7	19
	1,008	87 ± 10	12
	1,046	92 ± 7.0	9
	1,376	86 ± 15	15
	1,170	91 ± 6.6	15
	874	92 ± 7.2	14
	706	87 ± 11	11

81).

TABLE 9. SURVIVAL AND GROWTH OF SECOND GENERATION (F_1) FATHEAD MINNOWS AFTER 30 DAYS CONTINUOUS EXPOSURE TO CL-92,553.

Exposure level	Mean measured[a] concentration (µg/ℓ)		Survival (%)	Total length (mm) Mean ± S.D.	Wet weight (g)
1	47 ± 8.4	A	33	22 ± 2	0.103
		B	55	20 ± 3	0.084
2	21 ± 5.0	A	83	19 ± 3	0.058
		B	100	21 ± 2	0.078
3	11 ± 4.1	A	98	20 ± 2	0.074
		B	95	21 ± 2	0.082
4	5.9 ± 1.2	A	98	21 ± 2	0.089
		B	85	21 ± 2	0.089
5	3.0 ± 1.1	A	100	19 ± 2	0.065
		B	88	21 ± 2	0.085
	solvent control	A	90	22 ± 2	0.091
		B	100	21 ± 2	0.078
	control	A	100	19 ± 2	0.074
		B	88	20 ± 3	0.069

[a] Measured concentrations between test day 172 and 226.

TABLE 10. SURVIVAL AND GROWTH OF SECOND GENERATION (F_1) FATHEAD MINNOWS AFTER 30 AND 60 DAYS CONTINUOUS EXPOSURE TO CL-92,553.

Exposure level	30 Days				60 Days				
	Mean measured[a] concentration (µg/ℓ)		Survival (%)	Total length (mm) Mean ± S.D.	Mean measured[a] concentration (µg/ℓ)		Survival (%)	Total length (mm) Mean ± S.D.	Wet weight (g)
1	40 ± 8.0	A	70[a,c]	17 ± 4	42 ± 7.6	A	-	-	-
		B	48[a,c]	16 ± 4		B	-	-	-
2	22 ± 4.6	A	70	20 ± 3	23 ± 4.8	A	70	28 ± 8	0.252
		B	100	18 ± 2		B	100	23 ± 4	0.107
3	9.8 ± 2.6	A	98	19 ± 2	9.4 ± 2.4	A	93	24 ± 4	0.152
		B	95	22 ± 3		B	93	32 ± 4	0.305
4	6.8 ± 1.3	A	88	19 ± 4	6.6 ± 1.2	A	78	25 ± 4	0.151
		B	80	19 ± 3		B	80	26 ± 5	0.145
5	3.9 ± 1.7	A	98	20 ± 3	4.0 ± 1.5	A	90	27 ± 3	0.191
		B	95	17 ± 2		B	93	25 ± 4	0.158
	solvent control	A	90[b]	18 ± 4	solvent control	A	-	-	-
		B	98[b]	18 ± 3		B	-	-	-

TABLE 10. CONTINUED.

Exposure level	30 Days[a] Mean measured concentration (µg/ℓ)		Survival (%)	Total length (mm) Mean ± S.D.	60 Days[b] Mean measured concentration (µg/ℓ)		Survival (%)	Total length (mm) Mean ± S.D.	Wet weight (g)
	control	A	93	19 ± 2	control	A	90.	26 ± 3	0.153
		B	78	19 ± 4		B	73	28 ± 6	0.116

a
Mean measured concentration during period fry were exposed 30 days (test day 206-270).

b
Mean measured concentration during period fry were exposed 60 days (test day 206-295).

c
Fry from eggs spawned in solvent control (30 day exposure).

d
Fry from eggs spawned in high treatment of CL-92,553 (30 day exposure).

TABLE 11. CONCENTRATIONS OF CL-92,553 IN WHOLE FATHEAD MINNOWS DURING CONTINUOUS AQUEOUS EXPOSURE OF FIRST GENERATION FISH.

Exposure level	64 Days[a]		168 Days[b]		288 Days[b]	
	water[c] (µg/ℓ)	tissue (µg/g)	water[d] (µg/ℓ)	tissue (µg/g)	water[e] (µg/ℓ)	tissue (µg/g)
1	36 ± 9.7	45 ± 14[f] (4)[g]	36 ± 9.1	80 ± 29 (4)	38 ± 9.4	42 ± 15 (4)
2	21 ± 7.3	23 ± 6.8 (3)	18 ± 7.1	33 ± 11 (4)	19 ± 6.8	18 ± 5.4 (4)
3	7.7 ± 2.6	9.6 ± 1.2 (4)	7.1 ± 2.5	12 ± 1.6 (4)	8.0 ± 3.0	5.3 ± 4.0 (3)
4	4.8 ± 1.6	5.2 ± 1.8 (4)	4.5 ± 1.6	6.3 ± 1.2 (2)	5.1 ± 1.7	8.6 ± 5.1 (4)
5	2.4 ± 1.3	3.1 ± 0.97 (4)	2.1 ± 1.1	4.0 ± 2.7 (4)	2.7 ± 1.4	3.0 ± 1.2 (4)
	solvent control	<0.050 (4)	-	-	-	-
	control	<0.050 (4)	-	-	-	-

a
Analysis of whole fish tissue.
b
Values calculated from measured concentrations in carcass and viscera portions.
c
Mean measured concentrations between days 0 and 64.
d
Mean measured concentrations between days 0 and 168.
e
Mean measured concentrations between days 0 and 288.
f
Mean and standard deviation.
g
Number of samples analyzed given in parentheses.

TABLE 12. MEASURED CONCENTRATIONS OF CL-92,553 IN EGGS AND SECOND GENERATION FATHEAD MINNOWS DURING CONTINUOUS AQUEOUS EXPOSURE.

Exposure level	eggs		30 day fry		60 day fry	
	water[a] (µg/ℓ)	tissue (µg/g)	water[b] (µg/ℓ)	tissue (µg/g)	water[c] (µg/ℓ)	tissue (µg/g)
1	43 ± 8.1	12.2[d]	47 ± 8.4	44 ± 14[e] (4)	-	-
2	22 ± 5.0	4.1	21 ± 5.0	9.9 ± 8.0 (3)	23 ± 4.8	8.7 ± 1.9 (4)
3	9.8 ± 3.1	3.4	11 ± 4.1	4.5 ± 1.6 (4)	9.4 ± 2.4	7.1 ± 4.3 (4)
4	6.3 ± 1.3	3.0	5.9 ± 1.2	4.1 ± 1.8 (4)	6.6 ± 1.2	1.2 ± 0.41 (4)
5	3.7 ± 1.4	0.85	3.0 ± 1.1	1.5 ± 0.41 (4)	4.0 ± 1.5	4.3 ± 3.4 (4)
	control	-	-	-	-	0.46 ± 0.49 (4)

a Mean measured concentrations during spawning period (day 170-281).

b Mean measured concentrations between days 172 and 226 (period during which these fry groups were exposed).

c Mean measured concentrations between days 206 and 295 (period during which fry groups were exposed).

d Average of two determinations.

e Mean ± standard deviation (number of all samples).

TABLE 13. MEASURED CONCENTRATIONS OF CL-92,553 IN THE CARCASS AND VISCERA OF FATHEAD MINNOWS AFTER 168 DAYS AQUEOUS EXPOSURE AND AFTER 5, 10 AND 15 DAYS OF SUBSEQUENT DEPURATION IN UNCONTAMINATED WELL WATER.

Exposure level	Mean measured concentration in water (µg/ℓ) day 0-168	Mean measured concentration in tissue (µg/g)							
		exposure		depuration					
		168 days		5 days		10 days		15 days	
		carcass	viscera	carcass	viscera	carcass	viscera	carcass	viscera
1	36 ± 9.1	51 ± 7.7[a] (4)[b]	310 ± 180 (4)	0.39[c] (1)[d]	2.9 (1)	0.38 (1)	<2.5[e] (<1)	<0.062 (<1)	<0.46 (<1)
2	18 ± 7.1	22 ± 6.3 (4)	130 ± 66 (4)	<0.70 (<3)	1.2 (1)	0.15 (1)	2.0 (2)	<0.082 (<1)	<0.45 (<1)
3	7.1 ± 2.5	8.5 ± 1.2 (4)	36 ± 8.8 (4)	0.76 (9)	1.2 (3)	<0.19 (<2)	<0.33 (<1)	<0.050 (<1)	<0.22 (<1)
4	4.5 ± 1.6	4.8 ± 0.40 (2)	17 ± 7.1 (4)	0.34 (7)	1.1 (7)	0.30 (6)	<1.2 (<7)	<0.10 (<2)	<0.50 (<3)
5	2.1 ± 1.1	3.0 ± 2.3 (4)	12 ± 7.1 (4)	0.42 (14)	2.6 (22)	<0.06 (<2)	<0.39 (<3)	<0.050 (<2)	<0.39 (<3)

a Mean and standard deviation.

b Number of samples.

c All values during depuration are average of two samples.

d Percentage of 168 day tissue concentrations remaining.

e < values indicate one or both samples below minimum detectable (minimum higher for small sample weight, i.e., viscera).

TABLE 14. MEASURED CONCENTRATIONS OF CL-92,553 IN THE CARCASS AND VISCERA OF FATHEAD MINNOWS AFTER 288 DAYS AQUEOUS EXPOSURE AND AFTER 1, 2 AND 3 DAYS OF SUBSEQUENT DEPURATION IN UNCONTAMINATED WELL WATER.

Exposure level	Mean measured concentration in water (µg/ℓ) day 0-188	Measured concentration in tissue (µg/g)							
		exposure		depuration					
		288 days		1 day		2 days		3 days	
		carcass	viscera	carcass	viscera	carcass	viscera	carcass	viscera
1	38 ± 9.4	34 ± 16[a] (4)[b]	103 ± 58 (4)	34[c]	93	21	104	6.9	25
2	19 ± 6.8	14 ± 6.9 (4)	44 ± 14 (4)	21	48	4.4	23	<7.5[e]	24
3	8.0 ± 3.0	5.2 ± 3.6 (4)	17 ± 10 (3)	-[d]	-	-	-	-	-
4	5.1 ± 1.7	5.3 ± 1.4 (4)	29 ± 26 (4)	-	-	-	-	-	-
5	2.7 ± 1.4	2.4 ± 1.4 (4)	7.6 ± 3.6 (4)	-	-	-	-	-	-

[a] Mean and standard deviation.

[b] Number of samples.

[c] All values during depuration are average of two samples.

[d] No depuration of fish from these concentrations.

[e] < value indicates one sample below minimum detectable concentration.

Additional Test Data:

(1) Test methodology followed: U. S. EPA. 1971. Recommended Bioassy Procedure for Fathead Minnow (Pimephales promelas, Rafinesque) Chronic Tests.

(2) Duplicate tests were conducted for all five treatment concentrations and for solvent control and untreated control.

(3) Acetone was used as solvent for the initial 14 days of study. Excessive evaporation and crystallization of test material resulting from use of acetone solvent caused wide daily variations in measured test concentrations. Therefore, dimethylsulfoxide was used as the solvent for the remainder of the study (days 15-295).

(4) Twenty fry per treatment were used to determine survival from 0 to 60 days.

(5) Three males and seven females were used for egg production and hatchability determinations in each treatment.

(6) All measured biological parameters were analyzed statistically by analysis of variance. Data for percent survival and percent hatch were transformed to arc sin $\sqrt{\text{percentage}}$ prior to analysis.

Evaluation Category Rationale:

Test methods used in this study followed protocol recommended by EPA. However, the study was classified "Supplemental" because this reviewer's interpretation of the egg production and hatchability data (Table 8) differed from the conclusions presented by the investigator (see Discussion).

104.0 Hazard Assessment

104.1 Discussion

Prowl herbicide was accepted for registration in 1975 (see letter from R. J. Taylor to American Cyanamid Co. dated June 12, 1975) with the requirement that the registrant complete a chronic fish study within 2 years. Progress reports on this chronic fish study

fish study have been submitted (reviews by N. J. Cook, March 28, 1977, and R. W. Felthousen, July 10, 1976). This submission of the completed report is for final evaluation of the Prowl registrations.

Formulations of Prowl are presently registered for use alone or in tank-mixes with other herbicides on field corn, cotton and soybeans. Prowl is used in pre-emergent broadcast or pre-plant incorporated applications for control of undesirable vegetation in the above crops.

The investigator's interpretation of the egg production and hatchability data for fathead minnows continuously exposed to Prowl for a complete life cycle (Table 8) were questionable. The investigator concluded that there were no adverse effects on spawning of test fish at treatment concentrations of Prowl which were tested (i.e., $\leq$ 43 ppb). This conclusion was reached although the total number of eggs, eggs per spawn, and eggs per female exposed to 9.8 ppb were lower than those observed in all other treatments. These differences were ignored by the investigator because higher egg numbers were obtained from fish exposed to higher Prowl concentrations (22 ppb and 43 ppb). Yet there was no evidence offered to explain the reduced egg production observed at 9.8 ppb. Egg production by females in the 43 ppb treatment also appeared to be substantially lower than the control groups (Table 8). Although the mean number of eggs per spawn was similar between the 43 ppb treatment and the solvent-control group, the mean number of eggs per female was significantly lower ($P=0.04$, unpaired t - test) for the 43 ppb group (638 eggs per female) than among the solvent-control group (1273 eggs per female). The inconsistent results on spawning of test fish may be attributable to a relatively small number of females (3 to 7) used per treatment (Table 7) instead of 11-12 females per treatment as recommended in EPA protocol.

The investigator also reported that the mean percentages of eggs hatched from fish exposed to the 43 ppb and 22 ppb Prowl treatments were not significantly different from means of the controls and other treatments (Table 8). However, this reviewer checked the statistical comparisons and found evidence that hatchability data from both of the above treatments

were significantly lower than the solvent-control group. Individual unpaired t - tests using the arc sin √percentage transformation showed significantly lower percentages of eggs hatched for the 43 ppb treatment ($P < 0.01$) and for replicate = A of the 22 ppb treatment ($P = 0.048$). A second individual comparison using Chi-square tests (2 X 2 contingency table) showed significantly lower percentages of eggs hatched ($P < 0.01$) for both the 43 ppb and 22 ppb treatments. Therefore, the investigator should check his statistical analyses to confirm his initial conclusions. If the investigator maintains his original conclusion that no adverse effects on reproduction were observed during this chronic fish test for Prowl, the registrant must submit sufficient environmental fate data to demonstrate that the highest treatment level tested equals or exceeds the estimated concentration of Prowl in aquatic environments.

107.0 Conclusions

The registrant has satisfied the Environmental Safety data requirement for completion of a chronic fish study within 2 years of registration of Prowl herbicide. The study was conducted in compliance with recommended EPA protocol. However, the test results for the major fish reproduction parameters (egg production and egg hatchability) are difficult to interpret. The investigator's conclusion that fish reproduction was not adversely affected at the toxicant concentrations tested is questionable.

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