UNDATED

109301

DATA REVIEW NUMBER: (ES) X-1

TEST: Chronic Freshwater Fish Study

CHEMICAL: Pydrin (96%)

TEST SPECIES: Fathead minnow

REGISTRANT: Shell Chemical Co. (Test conducted by Bionomics, Aquatic Toxicology Lab)

DATE OF TEST: February 1978

ACCESSION NO. 97000 (Tab #9)

REVIEWER: L. A. Windberg

EVALUATION CATEGORY: Acceptable — ??

CATEGORY REPAIRABILITY: N/A

RESULTS: (1) Acute toxicity tests:

(a) 96-hr LC_{50} = 1.13 (0.92-1.38) ppb (nominal concentration)

(b) 21-day flow-through exposure to fry resulted in insignificant mortality at 0.06 ppb and 0.13 ppb, 65% and 80% mortality at 0.25 ppb, and 100% mortality (within 7 days) at 0.50 ppb and 1.00 ppb.

(2) Mean measured concentrations of SD-43775 in water during the 260 days of chronic exposure are summarized in Table 4 (attached). There were difficulties in maintaining the desired range of test concentrations. During the period between test days 42 and 63, measured concentrations of SD-43775 decreased as fish size and the quantity of food introduced in test tank increased. To reverse this trend, the concentration of the stock solution of SD-43775 was doubled on test day 63.

(3) 1st Generation (0-60 days): No significant differences in percentage hatch were observed among egg groups incubated in the control, solvent control, and mean measured concentrations of SD-43775 as high as 0.21 µg/ℓ was lower than in the remaining treatments.

Despite the poor survival of fry at this concentration, an analysis of variance indicated no significant differences due to treatment (P=0.05), apparently due to the variability between duplicate aquaria. The percentage survival of fish after 60 days exposure was nearly identical to that observed after 30 days in all aquaria. No significant differences due to treatment were observed in the total lengths of fathead minnows after both 30 and 60 days exposure.

(4) Survival and Growth of Adults:

The spawning period was completed between test days 168 and 260. A total of five mortalities in five separate aquaria occurred during this period. No significant differences due to treatment were observed in the total lengths and wet weights of male or female fathead minnows exposed 260 days in control, solvent control and concentrations of SD-43775 as high as 0.090 µg/ℓ (Table 8). Two male and three female survivors in the high concentration (0.21 µg/ℓ) were larger than fish in other treatments, apparently due to the reduced competition between fewer individuals.

(5) Reproductive Parameters:

The number of spawns per female and eggs per female from the few fish surviving exposure to 0.21 µg/ℓ was lower than in the remaining treatments (Table 9). Variability in spawning between duplicate aquaria of control, solvent control and 0.090 µg/ℓ SD-43775 makes the assessment of treatment related differences difficult. Allowing for differences between duplicate aquaria, spawning of fathead minnows does not appear to be adversely effected by exposure to concentrations of SD-43775 as high as 0.090 µg/ℓ. Eggs spawned by fathead minnows exposed to SD-43775 hatched in percentages similar to those spawned by control fish, including those spawned by survivors in 0.21 µg/ℓ (Table 10).

(6) 2nd Generation (0-30 days):

After hatching, all fry exposed to 0.21 µg/ℓ were killed during 30 days exposure (Table 10). No significant differences in survival or growth (total length, wet weight) were observed among second generation fry exposed 30 days in controls and concentrations of SD-43775 $\leq$0.090 µg/ℓ.

(7) Residue analyses (fish tissue):

Residue data from analyses of tissue samples were presented in Appendix I (attached). The investigator did not interpret these data in the report. This reviewer derived the following conclusions from the data:

(a) The bioaccumulation factor ranged from 1,500X to 10,000X in whole fish.

(b) Bioaccumulation in fathead minnows reached equilibrium by 63 days.

(c) Although bioaccumulation of pydrin in fathead minnows was relatively high, the evidence suggested that pydrin is not lypophilic (John Eaton, personal communication).

NOTE: Data on depuration will be submitted later by the registrant (Dr. Jack Bishop, personal communication).

Table 4 -- Measured concentrations of SD-43775 in water during 260 days of chronic exposure of fathead minnow.

Selected exposure concentrations (µg/ℓ)		Mean ± S.D.	Range	N^1[a]	N^2[b]
0.20	A	0.23 ± 0.14	0.013-0.70	39	0
	B	0.18 ± 0.11	0.047-0.63	39	0
	X	0.21 ± 0.12	0.013-0.70	78	0
0.10	A	0.093 ±0.094	0.018-0.56	39	0
	B	0.085 ±0.068	0.0075-0.35	37	2
	X	0.090 ±0.081	0.0075-0.56	76	2
0.050	A	0.059 ± 0.077	0.0038-0.40	38	1
	B	0.053 ± 0.076	0.0013-0.42	38	1
	X	0.056 ± 0.076	0.0013-0.42	76	2
0.025	A	0.032 ± 0.038	0.0025-0.20	39	0
	B	0.023 ± 0.026	0.0025-0.14	36	3
	X	0.028 ± 0.033	0.0025-0.20	75	3
0.013	A	0.021 ± 0.031	0.0017-0.19	38	1
	B	0.019 ± 0.025	0.0025-0.14	37	2
	X	0.020 ± 0.028	0.0017-0.19	75	3

[a]Number of samples used to compute mean.

[b]Number of samples below minimum detectable level.

Table 6 -- Survival and growth of first generation fathead minnow during chronic exposure to SD-43775.

Mean measured concentrations (µg/ℓ)		Hatching success	30 Day Old Fry		60 Day Old Fry	
			Survival (%)	Total Length (mm)	Survival (%)	Total Length (mm)
0.21	A	80	3	(1 fish)	3	(1 fish)
	B	82	15	23 ± 3	15	31 ± 5
0.090	A	66	40	21 ± 3	40	31 ± 3
	B	82	65	22 ± 3	65	30 ± 4
0.056	A	73	63	20 ± 3	63	28 ± 4
	B	66	73	23 ± 4	73	28 ± 4
0.028	A	90	40	20 ± 3	40	30 ± 4
	B	70	83	23 ± 2	83	30 ± 3
0.020	A	88	93	20 ± 3	93	27 ± 4
	B	85	50	24 ± 2	48	32 ± 3
solvent control	A	87	78	21 ± 2	75	28 ± 2
	B	86	85	22 ± 3	83	28 ± 4
control	A	70	68	21 ± 3	68	29 ± 3
	B	83	68	23 ± 2	68	29 ± 3

Table 8 -- Survival, growth and sexual maturity of F_o fathead minnow after 260 days exposure to SD-43775.

Mean measured concentrations (µg/ℓ)		Number of survivors			Total length (mm) ± S.D.		Wet weight (g)	
		males	females	immature	males	females	males	females
0.21	A							
	B	2	3	0	32 ± 1	64 ± 2	6.63	2.58
0.090	A	4	2	1	75 ± 5	60 ± 6	4.90	2.39
	B	3	6	0	73 ± 6	51 ± 4	5.49	1.28
0.056	A	3	6	0	70 ± 4	51 ± 2	4.76	1.29
	B	3	6	0	76 ± 2	58 ± 2	5.60	1.36
0.028	A	2	6	0	67 ± 0	54 ± 5	3.91	1.47
	B	4	2	0	74 ± 1	53 ± 2	4.59	1.39
0.020	A	4	4	1	70 ± 6	53 ± 5	3.94	1.35
	B	3	5	0	69 ± 3	55 ± 4	4.16	1.56
solvent	A	3	5	0	74 ± 2	53 ± 2	4.92	1.51
control	B	3	4	0	75 ± 7	54 ± 5	5.53	1.58
control	A	3	4	0	64 ± 9	53 ± 3	3.56	1.40
	B	3	6	0	70 ± 1	54 ± 5	4.13	1.49

Table 9--Egg Production of fathead minnows exposed to SD-43775.

Mean measured concentrations (µg/ℓ)		Total spawns	Total Eggs	Eggs per spawn	Spawns per female	Eggs per female
0.21	A	-	-	-	-	-
	B	4	615	154	1	205
0.090	A	7	1,238	177	4	619
	B	41	7,860	192	7	1,310
0.056	A	38	9,313	245	6	1,552
	B	27	6,375	236	5	1,063
0.028	A	42	9,591	228	7	1,599
	B	15	2,962	197	8	1,481
0.020	A	20	4,856	243	5	1,214
	B	33	5,470	166	7	1,094
solvent control	A	26	4,592	177	5	918
	B	20	2,235	112	5	559
control	A	22	3,526	160	6	881
	B	34	7,796	229	6	1,299

Table 10--Hatching success, survival and growth of second generation (F_1) fathead minnow after 30 days exposure to SD-43775.

Mean measured concentrations (µg/ℓ)		Hatching success (%) Mean ± S.D.	N[a]	30 Day Old Fry Survival	30 Day Old Fry Total length (mm) Mean ± S. D.	30 Day Old Fry Wet Weight (g)
0.21	A	78 ± 28[b]	2[b]	0[b]	-	-
	B	83 ± 15	4	0[b]	-	-
0.090	A	70 ± 27	7	78	23 ± 3	0.115
	B	85 ± 8	10	95	20 ± 2	0.083
0.056	A	82 ± 21	10	98	20 ± 2	0.080
	B	88 ± 7	10	93	20 ± 2	0.087
0.028	A	81 ± 26	10	100	20 ± 2	0.081
	B	71 ± 34	7	78	22 ± 3	0.091
0.020	A	77 ± 20	9	93	23 ± 2	0.091
	B	88 ± 9	9	100	19 ± 4	0.074
solvent control	A	86 ± 16	10	100	20 ± 2	0.093
	B	81 ± 18	8	78	22 ± 2	0.111
control	A	84 ± 17	7	68	22 ± 2	0.127
	B	85 ± 10	10	88	20 ± 2	0.073

[a]Number of egg groups incubated (50 eggs/group).

[b]Transfers from unexposed parents.

ANALYTICAL RESULTS

Analyses of Tissue Samples

Code No.	Sample	Target Conc. of SD 43775 in water, ppb	Amount (ug) of SD 43775 Added per liter of water (ppb)[a]	Exposure Interval, Days	SD 43775 Residue Found, ppm[c]	Bioaccumulation Factor
5	60 day Juvenile Fish	None	0	-	<0.01	
-	60 day Juvenile Fish	0.2 (.13)	0.2	-[b]	N.S.	-
1	" " " "	0.1 (.051)	-	63	0.24	4706X
2	" " " "	0.05 (0.25)	-	"	0.12	4800X
3	" " " "	0.025 (.015)	-	"	0.04	2667X
4	" " " "	0.013 (.006)	-	"	0.04	6667X
12	Whole Fish	None	0	-	<0.01	
-	Whole Fish	0.2 (.21)	0.4	-	N.S.	-
22	" "	0.1 (.09)	-	168	0.67	7444X
13	" "	0.05 (.056)	-	"	0.25	4464
19	" "	0.025 (.028)	-	"	0.11	3929X
16	" "	0.013 (.020)	-	"	0.07	3500X
11	Fish Muscle	None	0	-	<0.01	
-	Fish Muscle	0.2	0.4	-	N.S.	-
23	" "	0.1	-	168	0.37	4111X
14	" "	0.05	-	"	0.13	2321X
20	" "	0.025	-	"	0.1	3571X
17	" "	0.013	-	"	0.06	3000X

ANALYTICAL RESULTS

Analyses of Tissue Samples

Code No.	Sample	Target Conc. of SD 43775 in water, ppb	Amount (ug) of SD 43775 Added per liter of water (ppb)	Exposure Interval, Days	SD 43775 Residue Found, ppm	Bioaccumulation Factor
85	Whole Fish (Male)	None	0	-	<0.01	
88	" " (Female)	None	0	-	<0.01	
59	Whole Fish (Male)	0.2	0.4	260	1.3	6500X
60	" " (Female)	0.2	0.4	"	2.1	10,000X
61	" " (Male)	0.1	-	"	0.32	3,556X
64	" " (Female)	0.1	-	"	0.81	9,000X
67	" " (Male)	0.05	-	"	0.09	1,607X
70	" " (Female)	0.05	-	"	0.40	7,143X
73	" " (Male)	0.025	-	"	0.06	2,143X
76	" " (Female)	0.025	-	"	0.13	4,643X
79	" " (Male)	0.013	-	"	0.03	1,500X
82	" " (Female)	0.013	-	"	0.07	3,500X
29	Fish Eggs	None	0	-	<0.01	
-	Fish Eggs	0.2	0.4	-	N.S.	-
34	" "	0.1	-	-	0.08	889X
33	" "	0.05	-	-	0.06	1,071X
32	" "	0.025	-	-	0.02	714X
31	" "	0.013	-	-	0.02	1000X

a) From 0-63 days, 28 ul of 14 mg SD 43775/500 ml DMSO (28 ug/ml) was injected per gallon (3.875 l) of water to effect the high target concentration of 0.2 ppb. The 0.2 ppb water was further diluted to effect the lower target concentrations. However, water analyses indicated levels of approximately one-half the target concentrations. Thereafter, the amount of SD 43775 added was doubled (concentration of spiking solution doubled to 28 mg/500 ml DMSO) to bring the analytically measured water concentrations to target levels on day 63 and thereafter.

b) Three days exposure as embryos.

c) N.S. indicates that no sample was collected

ADDITIONAL TEST DATA:

(1) Test methodology followed: "Recommended Bioassay Procedures for Fathead Minnows (Pimephales promelas, Rafinesque) Chronic Tests" (U.S. EPA, 1971).

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

(3) Dimethyl sulfoxide was used as solvent.

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

(5) Three males and six females were used for egg production and hatchability determinations in each treatment. If less than six females were available, a maximum of four males were used 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 percentage prior to analysis. Significant differences were found by use of Duncan's Multiple Range Test. Effect levels noted below were statistically significant.

(7) Interpretation of test results: This reviewer has drawn the following conclusions from the test results reported by the investigator:

   (a) Hatching success was not affected at any treatment level (.02 to .21 ppb).

   (b) The effect level for survival of fry was >0.09 ppb<0.21 ppb.

   (c) Growth of test fish was not affected at any treatment level (.02 to .21 ppb).

   (d) The effect level for egg production (i.e. reproductive impairment) was >0.09 ppb<0.21 ppb.

   (e) Bioaccumulation factors ranged from 1,500X to 10,000X, but pydrin residues did not appear to be lipophilic.

EVALUATION CATEGORY RATIONALE:

Test methodology for this study was acceptable.

Duncan's multiple range test for significance

1. $SD = \sqrt{MS_e/r} = \sqrt{(SS_e/dfe)/r}$

SS_e = (1) 545278 (2) 1185.5 (3) 6048496 9.5

$dfe(all) = 7$ $r(all) = 2$

S'_D = (1) $\sqrt{(545278 \div 7)/2}$ (2) $= \sqrt{(1185.5 \div 7)/2}$ (3) $\sqrt{(6048496 9.5 \div 7)/2}$

= (1) 197.4 (2) = 9.2 (3) 2078.6

2. $n = dfe = 7$

3. range of means	2	3	4	5	6	7
tabular SSR	3.35	3.47	3.54	3.58	3.60	3.61
4. calculated LSR ($S_D \times SSR$)						
(1)	661.3	685	698.8	706.7	710.6	712.6
(2)	30.82	31.92	32.57	32.94	33.12	33.21
(3)	6963	7213	7358	7441	7483	7503

total eggs (3) X_3 X_4 X_7 X_5 X_2 X_6 X_1

7844 6276 5661 5163 4549 3413.5 307.5

X_3 diff X_1 only

total spawns (2) X_3 X_4 X_7 X_5 X_2 X_6 X_1

32.5 28.5 28 26.5 24 23 2

no sign. diff

eggs per female (1) X_4 X_3 X_5 X_7 X_2 X_6 X_1

1540 1307 1154 1090 965 738 102

X_1 sign diff from all

X_4 diff X_6

Arroyo - Pydrin
chronic fish/eggs per female
4/20/74
L. Turner

C.	
20	5.
	102.5 —
10506.	25
	61
	131
	964.5 —
119370.	25
	155
	106
	1307.5 —
59780.	25
	159
	148
	154 —
	348
	121
	109
	115 —
	360
	91
	55
	738.5 —
32220.	25
	88
	129
	109 —
43681.	
5.	52698990 2
	6.
	7.
	545278.
2583210.	857
3128488.	857

Arroyo - Pydrin
chronic fish/total spawns
4/20/74
L. Turner

C.	
	2.
	2. —
	2.
	7.
	41.
	24. —
	289.
	38.
	27.
	32.5 —
	30.25
	42.
	15.
	28.5 —
	182.25
	20.
	33.
	26.5 —
	42.25
	26.
	20.
	23. —
	9.
	22.
	34.
	28. —
	36.
1.	17699985 9
	6.
	7.
	1185.5
	119 6.
	2381.5

Arroyo - Pydrin
chronic fish/total eggs
4/20/74
L. Turner

C.	
	615.
	307.5 —
94556.	25
	1238.
	786 0.
	4549. —
10962721.	
	931 3.
	637 5.
	784 4. —
2157961.	
	95 91.
	296 2.
	6276.5 —
10985910.	25
	485 6.
	547 0.
	516 3. —
	9424 9.
	459 2.
	223 5.
	341 3.5 —
1388862.	25
	352 6.
	779 6.
	5661. —
4558225.	
1.	32958935 1
	6.
	7.
60484969.	5
68931575.	43
129416544.	9

Atrazine - Hydrilla
Chronic fish / 30 day old fry survival
1/20/78
L. Turner

3.
15.

9. 1
38.

40.
65.

52.5 2
151.25

63.
73.

68. 3
25.

40.
83.

61.5 4
461.25

93.
50.

71.5
461.25

78.
85.

81.5 6
111.25

68.
68.

68. 7
0.

3.409871663 F
6. DF num
7. DF den

2308. error Σ ss

6745.71286 treatment ss
9053.71286 total ss

$X_3 : X_4 = 0$	2	
$X_3 : X_5 = 6.5$	3	
$X_3 : X_6 = 15.5$	4	
$X_3 : X_7 = 54$	5	

$X_4 : X_5 = 6.5$	2	
$X_4 : X_6 = 15.5$	3	
$X_4 : X_7 = 54$	4	

$X_5 : X_6 = 9$	2	
$X_5 : X_7 = 52.5$	3	
$X_6 : X_7 = 43.5$	2	

① 0.6668

② 54

③

2	3	4	5	6
2.84	2.99	3.09	3.15	3.21
1.89	1.99	2.06	2.10	2.14

So X 6

X_1	X_2	X_3	X_4	X_5	X_6
9.49	9.34	7.31	6.32	5.69	5.02

P

$X_1 : X_2$	.15	2
$X_1 : X_3$	2.18	3
$X_1 : X_4$	3.17	4
$X_1 : X_5$	3.8	5
$X_1 : X_6$	4.47	6

$X_2 : X_3$	2.03	2
$X_2 : X_4$	3.02	3
$X_2 : X_5$	3.65	4
$X_2 : X_6$	4.32	5

$X_3 : X_4$	2	.99
$X_3 : X_5$	3	1.62
$X_3 : X_6$	4	2.29

$X_4 : X_5$	2	.63
$X_4 : X_6$	3	1.3
$X_5 : X_6$	2	.67

6 treatments

4 - 6 = 8

48 = A8

$MSe = \frac{SSe}{dfE} = \frac{2308}{7}$

$dfE = (48-1) - (4-1) = 44$

$(12-1)(3-1) = 33$

$MS_e = 329.7$

$S_{\bar{n}} = \sqrt{MSe / r}$

$= \sqrt{324.7/2}$

$= 12.8$

$LSD = t_\alpha \sqrt{2s^2/r}$

s^2 = Error mean square

r = sample size

t_α = α level two-tail T for error df

Anova

2	3	4	5	6	7
3.35	3.47	3.54	3.58	3.60	3.61

X 12.8

2	3	4	5	6	7
42.88	44.42	45.31	45.82	46.08	46.21

X_1	X_2	X_3	X_4	X_5	X_6	X_7
81.5	71.5	68	68	61.5	52.5	9

	P		P		P
$X_1 : X_2 = 10$	2	$X_1 : X_6 = 29$	6	$X_2 : X_5 = 10$	4
$X_1 : X_3 = 13.5$	3	$X_1 : X_7 = 72.5$	7	$X_2 : X_6 = 19$	5
$X_1 : X_4 = 13.5$	4	$X_2 : X_3 = 3.5$	2	$X_2 : X_7 = 62.5$	6
$X_1 : X_5 = 20$	5	$X_4 : X_4 = 3.5$	3		

7
2

Friday 1

$$SD = \sqrt{\frac{329.7}{2}} = 12.84$$

~~SD = 18.2~~

Error mean SS = 384.67

S_p = 13.87

DF_E = 6

3.68

288.5

DFE = 8

	2	3	4	5	6	7
	3.26	3.39	3.47	3.52	3.55	3.57
x12	39.12	40.68	41.64	42.24	42.6	42.84
difference						

8.5 6.8 61.5 9 7

71.5 6.8 52.5

52.5

43.5

21