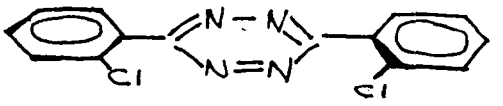
40363501

DATA EVALUATION

1. CHEMICAL:

chemical name: 3,6-Bis (2-chlorophenyl)-1,2,4,5-tetrazine
common name: Clofentezine (provisionally approved ISO as of 3/25/87)
trade name: Apollo SC
structure:



CAS #: 74115-24-5
Shaughnessy #: 125501

2. TEST MATERIAL: described below

3. STUDY/ACTION TYPE: submission of fish bioaccumulation study in support of registration

4. STUDY IDENTIFICATION:

Hill, R.W. et al. W70 CLOFENTEZINE: Determination of the Accumulation and Elimination of [^{14}C]-Clofentezine in Bluegill Sunfish (Lepomis macrochirus). dated 8/4/87. received EPA 10/5/87 under Acc. # 403635-01.

5. REVIEWED BY:

Typed Name: E. Brinson Conerly
Title: Chemist, Review Section 3
Organization: EAB/HED/OPP

E. Brinson Conerly
1/26/88

6. APPROVED BY:

Typed Name: Emil Regelman
Title: Supervisory Chemist, Reiew Section 3
Organization: EAB/HED/OPP

JAN 27 1988

7. CONCLUSIONS:

The study is unacceptable at this time. Further information may allow EAB to reconsider.

This submittal also contains a response to an Agency question regarding spiking of soil samples in a field study. EPA Acc. # 262273. The response indicated that samples were spiked immediately before extraction for analysis. This method is not automatically invalid, but is less representative of actual recoveries than is field spiking.

8. RECOMMENDATIONS:

The applicant company should do the following for the fish study:

If they believe the present study can be made acceptable, they must:

1. Demonstrate conclusively that the test fish did in fact reach equilibrium within the 14 day exposure period.
2. Give parameters of individual fish, such as length, weight, approximate age if known, for both test and control groups.
3. Explain the apparent lack of weight gain of the test fish.
4. Explain fully the tissue classifications used: viscera, flesh, and carcass.
5. Submit the results of the analyses for metabolites.

If they cannot provide satisfactory supplemental information outlined above, they must perform the required 28-day exposure Guidelines experiment and may wish to submit a protocol for approval before proceeding with yet another study.

Regarding the spiked samples, the applicant should provide any clarifying information on extractability of the compound vs contact time with the soil.

9. BACKGROUND:

This is a second study performed and submitted because samples from the prior study had been stored too long for metabolite analysis to be valid.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES:

A. Study Identification

Hill, R.W. et al. W70 CLOFENTEZINE: Determination of the Accumulation and Elimination of [^{14}C]-Clofentezine in Bluegill Sunfish (Lepomis macrochirus). dated 8/4/87. received EPA 10/5/87 under Acc. # 403635-01.

B. Materials and Methods

test materials -
unlabelled analytical grade clofentezine, 99.2% pure
tetrazine ring labelled [^{14}C]-clofentezine, sp. act. 140.5 uCi/mg, 99.6% pure (HPLC)

test solution - 73 mg labelled + 3 gm unlabelled materials described above in 500 ml acetone

saturation column - 25 ml increments of test solution and 50 gm increments of pumice were alternately added to a glass column until a total of 150 ml test solution and 300 gm pumice had been

applied. The acetone was then removed from the column by aerating for 70 hr (no liquid acetone was visible after the first hour).

test organism - bluegill sunfish, mean weight of 20 randomly selected fish, 0.88 gm [no range given] at the start of the study. [Non-exposed controls from the same batch are described elsewhere as also having a mean weight of 0.88 gm for 20 randomly selected fish (range 0.48 - 3.03 gm) and mean length of 32.1 mm (range 26 - 49.3 mm)]. On day zero of exposure test fish weighed an average of 1.26 gm, and control fish weighed an average of 1.27 gm. At day 14 of exposure, exposed fish had a mean weight of 1.33 gm, and controls had a mean weight of 1.53 gm.

test system - A dynamic system was used to produce a constant nominal concentration of 0.03 mg/L during the study. Light was a cycle of 12 hour light and 12 hour darkness. Flow rate was ca. 450 ml/min. Fish were exposed for 14 days and depurated for 7 days.

control system - as above, but the system contained no test material.

sampling protocol - 110 fish each were used in the experimental and control groups. Sampling was according to the attached protocol.

analytical method -

water analyses for radiolabelled compounds

LSC

HPLC

concentration of [^{14}C] in fish during equilibrium and exposure

oxidation followed by LSC

C. Reported Results

1. The mean measured concentration of clofentezine in the test vessel was 0.033 + 0.002 mg/l by LSC and 0.0.29 + 0.004 mg/l by HPLC.
2. Levels of radioactivity in the fish tissues had reached a plateau after 3 days.
3. Mean bioconcentration factors (BCF) for the edible, non-edible and viscera were 39x, 73x, and 2294x respectively. The overall (whole body) BCF was 248x.
4. Rapid elimination of [^{14}C] occurred during depuration. More than 96% of radioactivity was eliminated from the viscera, and >78% from the other tissues within 7 days. 93% of the radioactivity had been eliminated from the whole body of the fish within 7 days.

D. Study Author's Conclusions

1. The agreement of the results of the two water analyses indicates that little if any degradation of the compound occurred.
2. [^{14}C]-Clofentezine did not accumulate to any appreciable extent in either the edible or non-edible tissues.
3. Although a relatively high bioconcentration factor was obtained in the viscera, virtually all the accumulated residues were eliminated during the first 3 days of depuration.

E. Reviewer's Discussion and Interpretation of Study Results

1. It is not clear that the exposed fish had reached a steady state within the period of the experiment. **Mean** bioconcentration factors are fairly constant for the period of exposure -- i.e. a steady state was apparently reached within the first two days of exposure. However, **individual** values typically show threefold variation on a given experimental day. The significance of these two results is unknown. We need the parameters on the analyzed test fish such as weight, length, approximate age if known, in order to make the assessment.
2. The investigator's conclusion re the lack of degradation of the compound **in the water** is supported.
3. Residues do appear to be virtually eliminated within three days.
4. Control fish have gained approximately 20% of their original average weight over the 21 day course of the experiment, whereas test fish have only gained 5%. This may reflect a toxic effect of the pesticide. We need a clarification of this observation, and an explanation, if the investigator can furnish one.
5. Although a statement is made about sending samples for metabolite analysis, no results are reported on the metabolites. Only total radioactivity is reported. We need the values for metabolites, particularly since a major reason for rejection of the previous study was the lack of just such data.
6. The precise nature of the tissues analyzed is not clear from the terms used to describe them -- "flesh" and "carcass" require further explanation.

11. COMPLETION OF ONE-LINER: n. a.

12. CBI APPENDIX: attached

TABLE 3

Daily mortalities of bluegill sunfish observed during the exposure to clofentezine (initial 110 fish in both the treatment and freshwater control)

Date	Study day	Exposure/control fish removed for analysis	Mortalities exposure 0.03 mg/l	Mortalities freshwater control
	Acclimation		2	3
24.3.87	0		0	0
25.3.87	1	5	0	0
26.3.87	2		0	1
27.3.87	3	5	1	1
29.3.87	5		1	1
30.3.87	6		3	0
31.3.87	7	5	1	0
3.4.87	10	5	0	0
5.4.87	12		1	0
7.4.87	14	25	0	0
		Total 45	7	3
	DEPURATION PERIOD			
8.4.87	1	5	1	1
10.4.87	3	5	0	0
14.4.87	7	5	0	0
		Total 15		
	SURVIVING		40	43
	TOTAL FISH		110	110

TABLE 2

CLOFENTEZINE (Q153/B): SUMMARY OF TEST FISH ANALYSIS

(mg/kg wet weight)

	March			April				
Date	25.3.87	27.3.87	31.3.87	3.4.87	7.4.87	8.4.87	10.4.87	14.4.87
Day	(1)	(3)	(7)	(10)	(14)	(15)	(17)	(21)
						1st	3rd	7th
	A C C U M U L A T I O N					D E P U R A T I O N		
Viscera	59.4	69.5	69.9	143.3	67.7	76.3	3.9	3.9
Flesh	1.2	1.1	1.3	1.2	1.1	0.16	0.10	0.10
Carcass	1.7	2.6	1.5	2.5	2.2	0.99	0.58	0.60
Whole body	7.0	9.4	6.6	13.2	8.0	5.9	0.70	0.67
Viscera	76.9	151.0	88.0	86.1	59.4	20.0	4.5	2.9
Flesh	1.4	1.7	1.1	1.2	1.1	0.26	0.10	0.08
Carcass	2.0	2.7	2.1	2.3	1.9	0.79	0.67	0.55
Whole body	8.6	12.6	9.0	9.2	8.1	2.3	0.93	0.59
Viscera	23.4	103.8	57.7	30.0	71.1	13.2	5.6	1.8
Flesh	2.6	1.3	1.0	1.3	1.1	0.17	0.13	0.12
Carcass	4.7	2.7	1.8	2.1	2.2	0.90	0.64	0.57
Whole body	5.2	9.7	6.4	6.2	6.9	1.8	0.88	0.58
Viscera	64.6	43.9	83.9	74.7	63.6	7.4	4.3	2.7
Flesh	1.4	1.6	1.0	1.3	1.3	0.13	0.15	0.05
Carcass	2.5	4.8	2.0	2.3	2.2	0.73	0.79	0.41
Whole body	8.3	6.7	7.9	7.5	6.3	1.0	0.90	0.49
Viscera	78.6	76.6	104.2	64.0	80.4	3.4	5.0	3.8
Flesh	1.1	1.4	1.5	1.3	1.2	0.18	0.09	0.10
Carcass	2.2	1.8	2.4	2.2	2.1	0.85	0.54	0.56
Whole body	8.4	6.7	12.3	7.2	8.7	1.0	0.80	0.74
Means and ranges								
Viscera Mean	60.6	89.0	80.7	79.6	68.4	24.1	4.7	3.0
Range	(23.4-78.6)	(43.9-151.0)	(57.7-104.2)	(30.0-143.3)	(59.4-80.4)	(3.4-76.3)	(3.9-76.3)	(1.8-3.9)
Flesh Mean	1.5	1.4	1.2	1.3	1.2	0.18	0.11	0.09
Range	(1.1-2.6)	(1.1-1.7)	(1.0-1.5)	(1.2-1.3)	(1.1-1.3)	(0.13-0.26)	(0.09-0.15)	(0.05-0.12)
Carcass Mean	2.6	2.9	2.0	2.3	2.1	0.85	0.64	0.54
Range	(1.7-4.7)	(1.8-4.8)	(1.5-2.4)	(2.1-2.5)	(1.9-2.2)	(0.73-0.99)	(0.54-0.79)	(0.41-0.60)
Whole Mean body Range	7.5 (5.2-8.6)	9.0 (6.7-12.6)	8.4 (6.4-12.3)	8.7 (6.2-13.2)	7.6 (6.3-8.7)	2.4 (1.0-5.9)	0.84 (0.70-0.93)	0.61 (0.49-0.74)
Mean water concn	0.033	0.033	0.033	0.033	0.033	-	-	-
Bioconcn factor	227	273	255	264	230	-	-	-

JAN 27 1988

To: Dennis Edwards
Product Manager 12
Registration Division (TS 767C)

From: Emil Regelman, Supervisory Chemist
Review Section #3
Exposure Assessment Branch
Hazard Evaluation Division (TS 769C)

Thru: Paul F. Schuda, Chief
Exposure Assessment Branch/HED (TS 769C)

Attached, please find the EAB review of...

Reg./File #: 45639-EUP-GG

Chemical Name: Clofentezine

Type Product: Acaricide

Product Name: Apollo SC

Company Name: Nor-Am Chemical Company

Purpose: submission of studies in support of registration

Date Received: 10/16/87

Action Code: 711

Date Completed: _____

EAB #(s): 80046

Monitoring Study Requested: ____

Total Reviewing Time: 1.5 days

Monitoring Study Volunteered: __

Deferrals to: ___Ecological Effects Branch

___Residue Chemistry Branch

___Toxicology Branch