E. Bimson
Conerly

Shaughnessy Number: 125501

Date out of EAB: JUN 28 1988

To: Dennis Edwards/Portia Jenkins
Product Manager
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-RGL

Chemical Name: Clofentezine

Type Product: insecticide

Company Name: Nor-Am Chemical Company

Purpose: submission of additional data on fish bioaccumulation

Date Received: 03/15/88 Action Code: 111

Date Completed: 06/27/88 EAB #(s): 80782

Monitoring Study Requested: Total Reviewing Time: 2.5 d

Monitoring Study Volunteered:

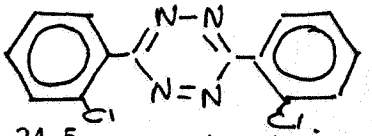
Deferrals to: Ecological Effects Branch

x Residue Chemistry Branch

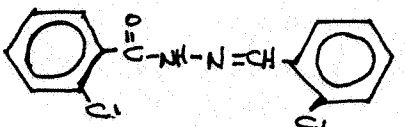
x Toxicology Branch

1. CHEMICAL:

chemical name: 3,6-bis (2-chlorophenyl)-1,2,4,5-tetrazine
common name: clofentezine
trade name: Apollo SC
structure:



clofentezine



2chlorobenzoic (2-chlorobenzylidine) hydrazide

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

2. TEST MATERIAL: n.a.

3. STUDY/ACTION TYPE: supplement to fish bioaccumulation study

4. STUDY IDENTIFICATION: Note that these three documents comprise a single study.

Hill, R.W. 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.

Arnold, D.J. and Newby, S.E. W78 CLOFENTEZINE: Bioconcentration of Clofentezine in Bluegill Sunfish: Analysis of radioactivity in Fish Tissues (Supplement to Original Report) performed by Schering Agrochemicals Limited, Essex, England. sponsored by Nor-Am Chemical Company, Wilmington, DE. dated 1/5/88, received EPA 1/12/88, under Acc.# 404679-12.

Hill, R.W. (W-70 Suppl 1) Determination of the Accumulation and Elimination of [^{14}C] Clofentezine in Bluegill Sunfish (Lepomis macrochirus) Supplement to Original Report. Nor-Am Chemical Company. dated 1/5/88, received EPA 1/12/88, under Acc.# 405522-01.

5. REVIEWED BY:

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

E.B. Conerly 6/27/88

6. APPROVED BY:

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

JUN 28 1988

7. CONCLUSIONS:

Although there are still deficiencies in this study, EAB will consider the data requirement satisfied. The submitted data, as well as other environmental fate data, suggest that bioaccumulation of the parent compound will not occur in the aquatic environment, based on short hydrolytic half life and low soil mobility.

8. RECOMMENDATIONS:

We defer to Toxicology and Residue Chemistry regarding whether degradates are a matter of concern and require additional studies.

9. BACKGROUND:

This compound is used in apple and pear orchards, and resulting pomace may be used as an ingredient in animal feed. Tolerances for pears, apples, apple pomace, and liver, milk, meat, and meat byproducts were proposed but not accepted as of 4/26/88.

The data base on the parent compound is as follows:

hydrolysis -- labile -- $t_{1/2}$ from 248.8 hr at pH 4.95 to 34.4 hours at pH 6.98 to 4.3 hr at pH 9.18 -- the principle product is 2-chlorobenzoic (2-chlorobenzylidine) hydrazide, which further degrades to 2-chlorobenzonitrile and 2-chlorobenzamide

photolysis --

aqueous -- labile -- $t_{1/2}$ < 7 days at pH 5

soil -- stable -- 85.9% parent remained after 31 days

soil metabolism -- moderately labile -- $t_{1/2}$ 4 -- 12 wks, products were CO_2 and a minor amount of 2-chlorobenzoic acid (interim report--final report is not in the file)

leaching -- no significant leaching of parent or degradation products

soil dissipation -- no leaching indicated -- $t_{1/2}$ 32.4 - 83 days

further information requested, not yet received

fish bioaccumulation -- discussed in this review

These data indicate a relatively short-lived, non-mobile compound. Particularly since the hydrolytic $t_{1/2}$ is so short, bioaccumulation in fish seems unlikely under environmental conditions.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES:

A. Study Identification

B. Materials and Methods

Submission 1:

The original submission is described in EBC review of 1/27/88 -- pH was ca. 7.

Submission 2:

extraction protocols

a) Viscera and edible tissue were macerated first with 50 ml acetone/dichloromethane (DCM) (90:10), then with 50 ml methanol/water (80:20); then filtered through paper. Residues were soxhlet extracted 18 hrs with methanol.

Storage containers were washed with either acetone or methanol to remove any adhering radioactivity, and added to the macerates before filtration.

Non-edible tissues were soxhlet extracted in methanol for 18 hours.

Total radioactivity was determined by LSC. Combined extracts from maceration and extraction were brought to a small volume by rotary evaporation for HPLC analysis.

b) Viscera, edible tissues, and non-edible tissues were separately soxhlet extracted for 18 hrs with DCM followed by acetonitrile/water (80:20), and analyzed as above.

analysis of unextracted radioactivity -- Following extraction, residual tissues were combusted and evolved $[^{14}C]O_2$ was trapped and counted.

analysis of 'polar' radioactivity in extracts

method 1 -- an aliquot was taken to dryness and the residue dissolved in 6M HCl and heated at 110°C for a total of 17 hrs. HPLC analysis showed that all radioactivity moved with the solvent front.

method 2 -- a dry residue of the HPLC eluant was prepared and dissolved in 48% HBr. This preparation was digested at ca. 110°C for 2 hours. Volatiles were trapped by means of a water-cooled condenser. The digested material was diluted with distilled water, and extracted with diethyl ether (DEE). The ether was partitioned with 0.1M NaOH and the ether portion discarded. The aqueous layer was then acidified and extracted 2x with DEE. The DEE extracts were dried, dissolved in acetonitrile/water (55:45) and analyzed by HPLC.

C. Reported Results

Submission 1:

1) Less than 6% of the total accumulated radioactivity was present in the edible tissue.
2) Approximately 50% of the radioactivity extracted from the edible tissue was characterized mainly as clofentezine.
3) Most of the accumulated radioactivity ... was present in the viscera and non-edible tissue.
4) Small amounts (3% or less) of ... metabolites, 2-chlorobenzoic (2-chlorobenzylidine)hydrazide and 2-chlorobenzamide were also detected.
5) ...attempts to characterize the remaining radioactivity by TLC and HPLC were [only] partially successful...[but it was] generally 'polar' material.
6) 93% of all tissue residues were eliminated ... during a 7 day depuration period.

Submission 2:

"Despite difficulties in handling the small amounts of materials involved, good overall recoveries of radioactivity were obtained from the two sets of fish tissues analyzed.

Recoveries of radioactivity in tissue extracts and combusted extracted tissue residues are shown in Table 2 [attached]. These figures were expressed as a percentage of the nominal radioactivity in the whole fish determined from a concentration of 7.6 mg/kg."

D. Study Author's Conclusions

Submission 1:

1) ... analysis... by both liquid scintillation counting and high performance liquid chromatography ... showed little, if any, degradation of the parent compound.
2) Plateau concentrations in the fish were achieved in approximately 3 days after which no significant further accumulation occurred.
3) [^{14}C]-Clofentezine did not accumulate to any appreciable extent in either the edible or non-edible fish tissues.
4) 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.

Submission 2:

"The accumulation phase of the...study resulted in a relatively low bioconcentration factor of 230x after 14 days. ...the majority (approximately 95%) was present in the non-edible (carcass) and viscera fractions, and only about 5% in edible tissues. Approximately 0.2 ug/g or less fresh weight of fish was ... unchanged clofentezine ... Despite rigorous 'clean-up' procedures, only small quantities of known clofentezine metabolites were characterized, the majority of radioactivity being present as 'polar' material, possibly conjugates in the viscera and non-edible tissues. However, ... it was shown that 93% of this accumulated radioactivity was eliminated from the whole body of the fish [during the depuration phase.] Consequently man is unlikely to be at risk from the uncharacterised residues."

E. Reviewer's Discussion and Interpretation of Study Results:

Submission 1:

1) Although conclusion 1 is not inconsistent with the results cited, two facts should be taken into consideration:

    a) The hydrolysis $t_{1/2}$ at the experimental ph is 34.4 hours at 22°C.
    b) The test aquarium is continuously receiving fresh clofentezine.

A more accurate description of the actual conditions might be that

the fish were exposed to essentially undegraded clofentezine, although hydrolysis was occurring during the experiment.

2) Conclusion 2 is not contradicted by the experimental results, but variability between individual specimens on a given sampling day is large. In the original review of this study, the applicant was asked for additional information. The response is discussed below in 10.3.

3) Conclusion 3 of the author is apparently supported.

4) Conclusion 4 of the author is apparently supported.

Submission 2:

The applicant's conclusions are consistent with the data as presented. However, the comment in a previous review remains: Since the data are so variable, interpretation is difficult, at best. The nominal concentration stated in 10.2C above is of unknown origin. The applicant should clarify.

Submission 3:

The following are EAB comments on Submission 1, Nor-Am responses, and EAB replies:

1) EAB comment --

Demonstrate conclusively that the test fish did in fact reach equilibrium within the 14 day exposure period.

Nor-Am response --

The data obtained for the whole body measurements days 1-14 inclusive have been subjected to one-way analysis of variance to determine if the values are different. The F-statistic for the 25 data points was 0.5 on 4 and 20 degree[s] of freedom. Therfore, there was no significant difference in the values at the 5% level. Therefore, it is concluded that equilibrium had been reached in this study.

EAB reply --

This explanation is acceptable (private communication, J. Blondell, 6/1/88).

2) EAB comment --

Give parameters of individual fish, such as length, weight, approximate age if known, for both test and control groups."

Nor-Am response --

Individual weight and length data were not determined on all fish at the start of the study, because such a practice causes undue stress...and can result in large numbers of random mortalities...

...0.88 g[m]...was the mean weight of the twenty fish left after [distribution] in the exposure and control vessels....[They] were used for ...control analyses....[60 control fish averaged 1.27 gm at day 0, and 30 control fish averaged 1.53 gm at day 14. 60 exposed fish averaged 1.26 gm at day 0, and 30 exposed fish averaged 1.33 gm at day 14.]

The fish...were...approximately 17/18 weeks old at the start of the study.

EAB reply --

These figures are the same as the ones upon which we based our previous comments, and are consistent with a lower weight gain for the experimental fish. See also below.

3) EAB comment --

Explain the apparent lack of weight gain of the test fish.

Nor-Am response --

[Full response is attached. The following is a paraphrased summary.]

i) The fish were deliberately fed a subsistence diet.
ii) Calculations reveal that experimental fish had an average [mean of three different sets of calculations] weight gain of 23% vs a 14% weight gain for controls.
iii) It has been demonstrated statistically that the fish did show an increase in body weight and length during the 14 day exposure.

EAB reply --

Please provide all the relevent <u>data</u> and include sample calculations to demonstrate the figures presented in the reply. The exposed fish must be shown to have been in comparable condition to the control fish when exposure and depuration took place. Note that a subsistence diet for the batch of fish may have resulted in a <u>below-subsistence</u> diet for some. If some fish actually lost weight during the exposure, the results, and therefore the interpretation of the results, may be highly questionable. Note also that although the mortalities of both groups is small, that of the control group is less than half that of the exposed group. The applicant has not offered an explanation. Attachment A to the submission cites a study not in Branch files and states that the 96 hr LC_{50} is greater than 0.25 mg/ml, vs the experimental concentration of 0.03 mg/l.

4) EAB comment --

Explain fully the tissue classification used: viscera, flesh and carcass.

Nor-Am response --

...viscera ...the alimentary canal (tract) and associated organs, excluding the kidney and heart

...flesh...a substantial portion of the muscle block (excluding skin and bone)
...carcass...the remainder of the fish not sampled for the above

EAB reply --
This deficiency is resolved.

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

12. CBI APPENDIX: attached

RIN 0633-94 CLOFENTEZINE REVIEW (125501)

Page____ is not included in this copy.

Pages 9 through 10 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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

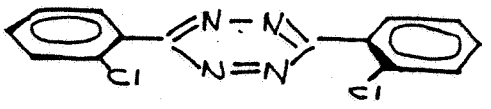
___Residue Chemistry Branch

___Toxicology Branch

11

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: F. 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

RIN 0633-94 CLOFENTEZINE REVIEW (125501)

Page_____ is not included in this copy.

Pages 16 through 17 are not included.

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