Pesticide Chemical No.: 125501

Date out of EAB: JAN 7 1987

To: Larry Schnaubelt
Acting Product Manager 12
Registration Division (TS 767)

From: Herbert L. Manning, Acting Chief HLM
Review Section #1
Exposure Assessment Branch
Hazard Evaluation Division (TS 769)

Attached, please find the EAB review of...

Reg./File # : 45639-RGL

Chemical Name: clofentezine

Type Product : acaricide

Product Name : Apollo

Company Name : Noram

Purpose :New chemical registration for use on apples; manufacturing use product

Date received: 4/18/86

Action Code(s): 110

Date completed: JAN 7 1987

EAB # (s) : 6560

days : 4.0

Deferrals to: _____ Ecological Effects Branch

_____ Residue Chemistry Branch

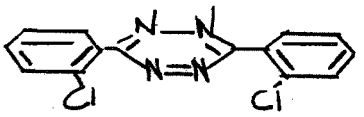
_____ Toxicology Branch

Monitoring study requested by EAB: /___/

Monitoring study voluntarily conducted by registrant: /___/

1. CHEMICAL

chemical name: 3,6-Bis (2-chlorophenyl)-1,2,4,5- tetrazine
proposed common name: clofentezine (provisionally approved ISO)
company code: NC 21 314
CAS no.: 74115-24-5
empirical formula: $C_{14}H_8C_{12}N_4$
molecular weight: 303.15
structural formula:



physical properties:
color- magenta
odor- odorless
physical state- crystalline solid
melting point- 179-182° C technical, 182-186° C pure active ingredient
specific gravity- technical, 1.52
solubility at 20° C-

water	1	mg/kg
acetone	0.5	g/100 ml
chloroform	5	g/100 ml
benzene	0.25	g/100 ml
ethanol	0.1	g/100 ml
hexane	0.1	g/100 ml
cyclohexane	170	mg/kg

2. TEST MATERIAL
as described in specific studies

3 STUDY/ACTION TYPE
request for registration as an acaricide (for mites) on apples and as a manufacturing use product

4. STUDY IDENTIFICATION
The Kinetics of the Hydrolysis of NC 21 314 under Acid, Neutral and Basic Conditions. Kelly, I.D. 20 January 1982. EPA acc. no. 262273
Characterization of the Hydrolysis Products of Clofentezine under Acid, Neutral and Basic Conditions. Smith, S. and I.D. Kelly. 4 June, 1985. EPA acc. no. 262273
The Photodegradation of (^{14}C)-Clofentezine in Water Under Natural Sunlight Conditions. Kelly, I.D. 29 April 1985. EPA acc. no. 262273
The Photodegradation of ^{14}C-Clofentezine on Soil. Kelly, I.D. 12 March 1985. EPA acc. no. 262273
The Degradation of NC 21314 in Three Soil Types under Aerobic, Sterile and Anaerobic Conditions. Leake, C.R. and D.J. Arnold. 15 August 1983. EPA acc. no. 262273
The Degradation of NC 21314 in a Clay Loam and Loamy Sand Soil at 15°C Leake, C.R. and D.J. Arnold. 25 April 1983. EPA acc. no. 262273
Laboratory Leaching Study with NC 21314 in Three Standard Soils from West Germany. Snowdon, P.J. 25 June, 1982. EPA acc. no. 262273
The Degradation and Leaching of NC 21314 in a Loamy Sand Soil. Leake, C.R. 12 July 1982. EPA acc. no. 262273
The Immediate Leaching of Apollo 50 SC in Three West German (Speyer) Soils. Leake, C.R. and D.J. Arnold. 29 Nov. 1985. EPA acc. no. 262273
The Leaching of NC 21314 in Four Soil Types Using Soil TLC. Leake, C.R. and D. Lines. 18 Jan, 1982. EPA acc. no. 262273
Residue Decline Study in Soil Following Application of NC 21314 to Bare Plots in Texas, 1980. Snowdon, P.J. 22 March 1982. EPA acc. no. 262273

Decline of Clofentezine Residues in Soil Following Orchard Treatment with the 50 SC Formulation in New York, USA, 1984. Snowdon, P.J. 26 Nov. 1985 EPA acc. no. 262273

Residue Decline Study in Soil Following Annual Single and Double Applications of NC 21314 (50W) at Shelford, U.K., Commencing 1981: Decline of Residues following 1981 Applications. Snowdon, P.J. 6 Oct., 1983. EPA acc. no. 262273

Residue Decline Study in Soil Following Annual Single and Double Applications of NC 21314 (50W) at Shelford, U.K., Commencing 1981: Decline of Residues following 1982 Applications. Snowdon, P.J. 10 June, 1983. EPA acc. no. 262273

Residue Decline Study in Soil Following Annual Single and Double Applications of NC 21314 (50W) at Shelford, U.K., Commencing 1981: Decline of Residues following 1983 Applications. Snowdon, P.J. 16 July, 1984. EPA acc. no. 262273

Summary of Residue Decline Studies in Soil following Annual Single and Double Applications of Clofentezine (50W) at Shelford, UK. between 1980 and 1983. Snowdon, P.J. Sept. 1985. EPA acc. no. 262273

Determination of the Accumulation of NC 21314 in Bluegill Sunfish (Lepomis macrochirus) using a Dynamic Test System. Hill, P.W., J.E. Caunter, and E. Gillings. Dec. 1982. EPA acc. no. 262273

5. REVIEWED BY:

Typed Name: E. Brinson Conerly
Title: Chemist, Review Section 1
Organization: EAB/HED/OPP

E. Brinson Conerly
1/6/87

6. APPROVED BY:

Typed Name: Herbert L. Manning
Title: Acting Chief, Review Section 1
Organization: EAB/HED/OPP

Herbert L. Manning
JAN 7 1987

7. CONCLUSIONS:

Hydrolysis of Clofentezine yields 2-chlorobenzoic (2-chlorobenzylidine) hydrazide, resulting from opening of the tetrazine ring. This compound further degrades to 2-chlorobenzonitrile and 2-chlorobenzamide. Half-lives range from 248.8 ~~days~~ hours at pH 4.95 through 34.4 ~~days~~ hours at pH 6.98 to 4.3 ~~days~~ hours at pH 9.18.

Aqueous photodegradation in pH 5.05 acetate buffer yielded principally 2-chloro benzonitrile (74.6% in 31 days), with lesser amounts of 2-chlorobenzaldehyde (8.3%), parent compound (5.7%), and 2-chlorobenzamide (1.3%) also present. Dark controls had mostly parent compound (58.7%) with lesser amounts of 2-chlorobenzaldehyde (12.8%), 2-chlorobenzonitrile (6.4%), and 2-chlorobenzamide (4.3%). The principal photoproduct is therefore 2-chlorobenzonitrile. The half-life under these conditions was not formally derived, but is < 7 days.

Soil photodegradation is slow, yielding small amounts of 2-chlorobenzonitrile and soil bound material (85.9% parent, 5.6% bound, and 5.5% 2-chlorobenzonitrile after 31 days.) Dark controls showed no appreciable degradation.

Two soil metabolism studies were in general agreement with each other. Principal radioactive products were CO_2 and bound material. Half lives ranged

from 4-8 weeks in one study and 9-12 weeks in the other.

Four leaching studies, including one TLC, two unaged column, and one aged column study all indicated that no significant leaching of clofentezine or degradation products occurred.

Soil dissipation studies on bare ground and on soil from a treated apple orchard again indicated no leaching. The projected half-life of clofentezine ranged from 32.4 - 83 days for this group of studies.

A fish accumulation study on this compound showed a bioaccumulation factor of 430 in whole fish, with 94% clearance within seven days after exposure was stopped. However, this study has serious deficiencies in that separate values for inedible and edible portions were not given.

8. RECOMMENDATIONS:

Data requirements for this use pattern, and their status, are as follows:

hydrolysis	satisfied
aqueous photolysis	satisfied
soil photolysis	satisfied
anaerobic soil metabolism	not satisfied, but see below
aerobic soil metabolism	not satisfied, but see below
leaching	satisfied, but see below
soil dissipation	satisfied
fish accumulation	not satisfied, but see below

For anaerobic and aerobic soil metabolism, the applicant should submit the pesticide treatment history of the test soil. For the leaching studies, the cation exchange capacity of the soils must be provided. For Study 10.8 "The Degradation and Leaching of NC 21314 in a Loamy Sand Soil", we need the amounts of specific compounds found, and details of the rationale for the correction of the CO_2 evolution. We need details of the sample treatment vs the "spiked" treatment in the orchard soil dissipation study. For the fish accumulation study, specification of the site of radiolabel and data on analyses for parent compound and metabolites in specific portions of the fish is required, as well as a clarification of the feeding rate.

9. BACKGROUND

This application is for use of the pesticide on apple orchards and as a manufacturing use product.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES

10.1

A. STUDY IDENTIFICATION

The Kinetics of the Hydrolysis of NC 21 314 under Acid, Neutral and Basic Conditions. Kelly, I.D. FBC report 20 January 1982. Study no. 31J. Report no. METAB/82/7. EPA acc. no. 262273

B. MATERIALS AND METHODS (Protocols)

test materials- NC 21 314 labeled with ^{14}C in the 3 and 6 positions of the tetrazine (central) ring, sp. act. 84.5 mCi/gm, radiopurity by TLC

(two systems) 99.4%, by HPLC 98.4% (rechecked at completion of the experiment by HPLC @ 98.2%)

test buffers-

purchased- pH 4.95- phthalate (0.1M KHP, 3.728 gm/L KCl, 50 ml N/1 KOH)
pH 6.98- phosphate (0.02 M KH_2PO_4, 0.03M Na_2HPO_4, 0.02M NaCl)
pH 9.18- borax (0.01M $Na_2B_4O_7.10H_2O$, 0.01M NaCl)

buffers were sterilized by heating in a boiling water bath for 20 min, and repeating the process after 24 hours.

test protocol- the tests were performed in glassware sterilized by heating for 2 h to 180C°.

Solubility of the compound was determined as a necessary preliminary to the hydrolysis study. 0.5 mg test material was dissolved in 1 ml acetone which was dispersed in 99 ml buffer @ pH 4.95. The resulting dispersion was shaken 16 h @ 22°C, spun @ 12,000 g for 15 min. and filtered. The concentration of the test material in the filtrate was determined by LSC to be ca. 0.029 mg/l

hydrolysis buffer solutions- 75 ml aliquots of sterilized buffer in 100 ml flasks were equilibrated in the dark at designated temperatures. (pH 4.95 and 6.98 were done at 22°C ± 1° and 38°C ± 1°; pH 9.18 was done at 22°C ± 1° and 10°C ± 1°.)

hydrolysis test solutions- approximately 0.014 mg/l and 0.026 mg/l in the above buffers and 1% in acetone.

analysis methods-

method 1- hydrolysis was stopped at sampling times by addition of 20 ml dichloromethane (DCM). The organic phase and 2 subsequent 20 ml DCM washings were dried by passage over an anhydrous Na_2SO_4 column, reduced *in vacuo* to 2-3 ml, and blown to dryness under an N_2 stream. This preparation was then redissolved in 0.25 ml methanol. Three aliquots of 0.01 ml were analyzed by LSC.

Method 2- hydrolysis was stopped at sampling times by passage through a C_{18} Sep-pak column prewashed with 5 ml 1,2 dimethoxyethane (DMOE) and 10 ml distilled H_2O. pH 9 samples were acidified with 2 ml 1M HNO_3 before column passage. Following a 10 ml distilled H_2O rinse, the column was eluted with 2 ml DMOE. 3 aliquots of 0.1 ml each were analyzed by LSC.

HPLC analysis

reference standards-

3,6-bis-(2-chlorophenyl)-1,2,4,5-tetrazine
2-chlorobenzoic (2-chlorobenzylidine) hydrazide
2,5-bis-(2-chlorophenyl)-1,3,4-oxadiazole
N,N'-bis-(2-chlorobenzoyl)-hydrazine
2-chlorobenzoic acid

Duplicate samples were analyzed at zero and sampling times until more than 50% of the starting material had been hydrolyzed.

Results were plotted, and were subjected to linear regression to establish best fit lines for each treatment.

C. REPORTED RESULTS

Rate constants, half lives and activation energies are reported in the attached table (table 10.1). To summarize, the half-lives were 248.8 hr at pH 4.95, 34.4 hr at pH 6.98, and 4.3 hr at pH 9.18 at 22° C.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

The hydrolysis reaction of clofentezine is first order, with the stability of the compound decreasing with increasing alkalinity and also with increasing temperature.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

The determination of the hydrolysis half lives, rate constants, and activation energies appear to be correct for the given conditions. Products were not determined in this experiment, but in a subsequent one (study 10.2, q.v.). Minor differences between the author's figures and this reviewer's do not invalidate the study, and the study is acceptable.

10.2

A. STUDY IDENTIFICATION

Characterization of the Hydrolysis Products of Clofentezine under Acid, Neutral and Basic Conditions. Smith, S. and I.D. Kelly FBC report 4 June 1985. Study no. 61J. Report no. METAB/85/11. EPA acc. no. 262273

B. MATERIALS AND METHODS (Protocols)

test buffers- as in 10.1 except that pH 5 buffer was used both at full strength and at a 20/1000 dilution
test solution-- approximately 0.029 mg/L, concentration accurately determined by LSC
test protocol-- as in 10.1. pH 9.2 hydrolysis temperature was 22± 1° C, and pH 5 and 7 temperature was 38± 1° C. Reactions were carried out for 1.5 half lives, and were terminated by extraction 4x with ethyl acetate (EtOAc). Each extracting volume of EtOAc was approximately 1/5 the aqueous volume. The combined organic extracts were dried with pentane/$NaSO_4$, reduced by rotary evaporation to c. 1 ml @ 30° C. This residue was brought to 1-2 ml with acetonitrile and an aliquot counted by LSC to determine recovery. A measured aliquot was subjected to HPLC analysis. The remainder was concentrated to 50 ul @ 30° C in a stream of N_2. After removal by centrifugation of a buffer precipitate in the pH 9.2 extract, the preparations were subjected to LC/MS and GC/MS for identification of the compounds present in the hydrolyzate.

C. REPORTED RESULTS

The major hydrolysis product after approximately 1 1/2 half-life reaction times under all conditions was determined to be 2-chlorobenzoic(2-chlorobenzylidene)hydrazide. Other, relatively minor, products were 2-chlorobenzamide and 2-chlorobenzonitrile. (See attached data, table 10.2.)

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

Three major compounds are formed in the hydrolysis of clofentizine at acid, neutral, and basic pHs in aqueous conditions. No other compound occurred in amounts greater than 5%.
The major product in all three cases is 2-chlorobenzoic(2-chlorobenzylidene) hydrazide. Other compounds, at less than 10% each, are 2-chlorobenzonitrile and 2-chlorobenzamide.

98% (or more) radioactivity was extracted into the EtOAc fraction. Recovery was $\geq$ 96% at each stage in the isolation procedure (except for an experiment requiring an additional transfer to counting vials, recovery 92.7%.)

Less clofentezine was hydrolyzed in experiment 3 than expected, so the hydrolysis was repeated with "full strength" buffer. Results were similar to those from experiment 3, but with somewhat more conversion of clofentezine.

Identity of peaks was confirmed by GC/MS and LC/MS using unlabelled standards.

A hydrolysis profile is proposed (table 10.2 a, q.v.), with the apparent rate-limiting step the opening of the tetrazine ring.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS

The material balance is presented stepwise, and shows excellent results. Note that overall recovery is apparently better than 90% in all cases.

The radio and UV tracings are hard to examine, due to overlap of the lines but the peaks seem not quite to coincide in the two media. The author attributes this slight discrepancy, also found in another study, to differences in detector configuration.

The study was performed in an approximately saturated solution.

This study together with the previous one satisfies the requirements for hydrolysis.

10.3

A. STUDY IDENTIFICATION

The Photodegradation of ^{14}C-Clofentezine in Water under Natural Sunlight Conditions. Kelly, I.D. FBC Report 29 April 1985. Study no. 52J. Report No. METAB/84/15. EPA acc. no. 262273.

B. MATERIALS AND METHODS

test materials

^{14}C clofentezine labelled in the carbons of the tetrazine rings
spec. act. 47.7 uCi/mg, 97.5% purity by HPLC
unlabelled clofentezine, 98.4% $\pm$ 1% pure
authentic standards for clofentezine degradates
2-chlorobenzoic (2-chlorobenzylidene) hydrazide
2,5-bis-(2-chlorophenyl)-1,3,4-oxadiazole
N,N'-bis-(2-clorobenzoyl)-hydrazine
2-chlorobenzamide
2-chlorobenzoic acid
2-chlorobenzonitrile
2-chlorobenzaldehyde
2-chlorobenzyl alcohol

apparatus-- all glass apparatus was heat sterilized as in 10.1.
buffer solution-- sodium acetate (0.1M, pH 5.05), sterilized as in 10.1

stock solutions-- 0.94 mg/ml and 0.097 mg/ml solutions of ^{14}C clofentezine in acetonitrile and 2 mg/ml unlabelled clofentezine in acetonitrile

test protocol--

test solution-- 250 ml sterile buffer added to each of eighteen 250 ml borosilicate glass conical flasks

eight masked with black adhesive tape-- dark controls

eight unmasked-- exposed samples

two unmasked, analyzed immediately-- "day zero"

treatment-- the experiment was initiated by adding 0.65 ml of the 0.097 mg/ml ^{14}C clofentezine to each flask, for a concentration of 0.25 mg/L. Exposure was on a building roof at Chesterford Park Research Station from 12 Aug,'83 to 12 Sept,'83. In addition, "high level" flasks containing 1 L acetate buffer, 0.5 ml of the 0.94 mg/ml ^{14}C clofentezine and 2 ml of the 2 mg/ml unlabelled clofentezine were exposed at the same time.

Duplicate samples from dark controls and exposed samples were taken at day 0, 10, 18, 24, and 31 days after treatment. "High level" samples were incubated an additional 8 days (a total of 39 days.)

analytical method

Samples were extracted 3x with 75 ml each of dichloromethane (DCM). The organic extracts were combined, dried over Na_2SO_4 or $MgSO_4$ and concentrated to 10 ml. Compounds were then characterized by two-dimensional TLC, and by HPLC following removal of the DCM and redissolving in acetonitrile.

C. REPORTED RESULTS

The recovery of organic and aqueous soluble radioactivity indicated no loss of activity over time (mean total recovery= 95.0% $\pm$ 4.2%).

Aqueous soluble fractions increased with time, 9.9% for "exposed samples" and 13.6% for dark controls.

Organic fractions were not successfully analyzed by TLC due to the rapid loss of volatiles. Radioactivity was not lost during concentration by rotary evaporation-- concentration for TLC produced a mean recovery of 96.9 $\pm$ 4.4%, and for HPLC 97.9 $\pm$ 3.9%. HPLC analysis showed complete recovery of activity, and all major peaks co-chromatographed with one of the standards. All other peaks were less than 5% of the eluted radioactivity. Results are summarized in the attached table (table 10.3)

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

Major products after 31 days exposure were, in descending order, 2-chlorobenzonitrile (74.6%), 2-chlorobenzaldehyde (8.3%), 2-chlorobenzamide (1.5%) and clofentezine (5.7%). In dark controls, the major products were clofentezine (58.7%), 2-chlorobenzaldehyde (12.8%), 2-chlorobenzonitrile (6.4%), and 2-chlorobenzamide (4.3%). A further minor product of photodecomposition was 2-chlorobenzoic acid (3.1%). Traces of this compound were found in the dark-sample aqueous extracts, but most of the activity

present in these samples was not identified during this study.

To summarize, photodecomposition products accounting for all but 7% of the recovered radioactivity have been identified. All but 5% of the radioactivity in the organic extract from the dark controls has been accounted for. 17.8% of the activity in the dark control, found in the aqueous extract, remained unidentified in this study.

The primary difference in the two pathways is the production of 2-chlorobenzonitrile in the light-exposed samples. The identity of this and the other major product, 2-chlorobenzaldehyde, have been confirmed by GC-MS. Other products were present in quantities too small for MS confirmation of their structures.

Theoretical rate constants and half-lives were not derived, since the light conditions varied greatly over the exposure period.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS

The qualitative conclusions of this study are supported, and the author has declined to attempt quantitative conclusions. Although the applicant did not so state, the aqueous photolysis half-life is clearly less than 10 days, the first sampling interval, under the described conditions, which this reviewer would classify as cool temperature and moderate sunlight. More intense light or warmer temperature would likely result in still shorter photolytic half-life. The study is acceptable.

10.4

A. STUDY IDENTIFICATION

The Photodegradation of ^{14}C-Clofentezine on Soil. Kelly, I.D. FBC report 12 March 1985. Study No. 53J. Report no. METAB/84/17. EPA acc. no. 262273

B. MATERIALS AND METHODS

test material-- ^{14} Clofentezine labelled in the tetrazine ring, sp. act. 47.4 uCi/mg, purity 97.5% by HPLC, prepared as an 0.94 mg/ml solution in acetonitrile (standardized by LSC.)

soil treatment-- oven-dried sandy loam soil (4 hr at 105° C) was sieved to <1 mm. Eighteen 15 gm portions were placed in separate 9 cm borosilicate glass petri dishes in a layer 2.5-5 mm deep. Eight dishes masked with adhesive black tape were the dark controls. Eight unmasked dishes were the photodecomposition samples, and the remaining two were the "day zero" samples, analyzed immediately. The experiment was started by the addition of 0.075 ml of the 0.94 mg/ml solution in an even layer over the soil surface (a concentration of 4.7 mg clofentezine/kg soil.) The samples were exposed on an unshaded rooftop in the Chesterford Park Research Station between 12 August 1983 and 12 September 1983.

sampling-- duplicate samples were taken on day 10, 18, 24, and 31.

analysis of samples-- the treated soil was moistened with 3 ml distilled H_2O and soxhlet extracted with acetone for 16 hr. The soxhlet extracts were then concentrated to c. 2 ml by rotary evaporation @ 35°C and 400 mbar and resuspended in 10 ml acetone.

TLC was carried out in two different solvent systems (chloroform:

methanol 98:2) and toluene:ethyl acetate:ethanol:acetic acid (80:5:10:0.5) Reference standards were located by UV inspection.

Radioactivity was detected by autoradiography and quantified by LSC of the removed spots. The samples were also analyzed by HPLC, with reference standards located by UV absorption. Radioactivity was detected and quantified by use of a radioactivity monitor. Quantification and recovery were verified by LSC.

Bound radioactivity was determined by combusting subsamples of extracted soil with added glucose and trapping the resulting $^{14}CO_2$ for LSC.

identification of products-- combined extracts were diluted with an equal volume of H_2O and extracted 3x with hexane. The combined hexane extracts were then dried over anhydrous Na_2SO_4, filtered, and evaporated (rotary evaporation @ 300 mbar and 30°C.) This material was then blown almost dry in N_2, resuspended in hexane, sonicated, centrifuged @ 3000 rpm for 2 minutes, and passed over a hexane-washed silica column. The material was then serially eluted with 10 ml dichloromethane:hexane (1:4 v/v) and 10 ml DCM:hexane (3:2 v/v). These extracts were combined, concentrated and subjected again to the silica column clean-up. Most of the radioactivity was then in the 3:2 fraction. For further purification, this fraction was concentrated to dryness, resuspended in acetone, and subjected to HPLC. The combined radioactive fractions were diluted with H_2O and partitioned with DCM, dried over anhydrous Na_2SO_4, concentrated to dryness and subjected to MS analysis.

C. REPORTED RESULTS

There was complete recovery of radioactivity in both dark and light samples, with a greater amount of bound activity in the photodecomposition samples (8.8% in the light compared to 3.1% in the dark after 31 days.) TLC analysis proved unsatisfactory due to problems of incomplete recovery and plate streaking. HPLC gave satisfactory quantitative results (mean recovery 98.0% $\pm$ 3.2%.)

In dark samples no significant degradation occurred (clofentezine >96% of the recovered radioactivity in all samples.) In light samples, 85.9% of the recovered radioactivity was parent compound after 31 days along with 5.5% 2-chlorobenzonitrile and 8.6% bound.

Half-lives and rate-constants were not derived, due to large variations in temperature and sunlight during the experiment.

Identity of the HPLC-purified material as clofentezine was confirmed by MS analysis. See Table 10.4 for details.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

Clofentezine is not degraded on soil in the dark during a 31-day period. 14% is degraded when exposed to light for the same period under the same climatic conditions, with the only identified degradation product being 2-chlorobenzene

and the rest bound to the soil.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS

The conclusions of the applicant are supported by the data-- the compound is relatively stable to photodegradation on soil, with a half-life greater than 31 days under the conditions of exposure, which this reviewer would classify as cool temperature, moderate light. The study is acceptable.

10.5

A. STUDY IDENTIFICATION

The Degradation of NC 21314 in Three Soil Types under Aerobic, Sterile and Anaerobic Conditions. Leake, C.R. and D.J. Arnold. FBC report 15 August 1983. Report no. METAB/83/31. EPA acc. no. 262273.

B. MATERIALS AND METHODS

test material--
^{14}C clofentezine labelled in the tetrazine ring, sp. act. 86.4 uCi/mg, radiochemical purity 98.1% by TLC
unlabelled NC 21 314, purity > 98.0%
stock solution-- 5.8 mg ^{14}C clofentezine + 11 mg unlabelled clofentezine in 80 ml dichloromethane
test soils-- English--

soil type	Organic Matter	pH	% sand coarse/fine		% silt coarse/fine		%clay	moisture capacity
clay-pasture	4.5%	6.6	35.13	6.58	2.80	3.15	49.23	114%
loamy sand-cultivated	1.9%	6.5	29.83	38.88	6.47	12.35	1.36	48%
clay loam-woodland	14.7%	6.2	19.97	10.12	9.78	10.75	26.78	113%

T½
4 wk.
6 "
8 "

soil preparation-- the soils were sieved to < 2 mm, and the moisture holding capacity and moisture content determined (zero suction, Hilgard Cup technique). Samples (65 gm dry soil equivalent for the clay and 50 gm for the loamy sand and clay loam) were weighed into 500 ml conical flasks. "Sterile" samples were autoclaved for 15 minutes at 15 psi.
experimental treatment-- 0.5 ml of stock solution was added to each flask, the solvent allowed to evaporate, and the moisture content adjusted to 50% of the moisture holding capacity with deionized H_2O.

Flasks were incubated in the dark (25°C ± 2°C) with circulation maintained by blowing a stream of moist CO_2-free air across the surface of the soil. Volatile products were trapped by passing the effluent air from each flask through two flasks containing 2-methoxy-ethanol:ethanolamine 4:1, which were sampled and replaced at intervals during the incubation. Moisture content of the soil was checked and adjusted as needed.

After 30 days aerobic incubation, some of the flasks were flooded,

purged with N_2 and incubated anaerobically, with trapping done as described above.

Incubation was carried out for as long as 1 year for aerobic samples, and 60 days after flooding for anaerobic samples. Sterile soils were incubated for 30 days maximum.

analyses— samples were analyzed in triplicate on each day of sampling. To remove radioactivity from the soil, samples were subjected to soxhlet extraction for three hours, first with dichloromethane, then with methanol:H_2O (9:1). Some were also subjected to a more severe extraction with acetonitrile:H_2O (80:20) for 18 hrs. Radioactivity was determined by LSC.

Extracts were concentrated by rotary evaporation under vacuum at 30°C, and analyzed by TLC against authentic standards in two solvent systems, toluene:ethanol:ethyl acetate:glacial acetic acid (80:10:5:0.5), and chloroform:methanol (49:1). Reference standards were located by UV inspection; autoradiograms were used to locate radioactivity.

Bound activity was determined by combustion of previously extracted soils with added glucose, and LSC quantification of the released $^{14}CO_2$.

Volatile products were quantified by the attached analytical scheme. (See table 10.5.a.)

C. REPORTED RESULTS : details in tables 10.5.b attached

overall recoveries:
aerobic-- 99.5%
sterile-- 103%
anaerobic— 91.3%

Volatile products from aerobic soils were determined to be almost all $^{14}CO_2$, and ranged from 25-56% of the total applied radioactivity after one year's incubation. Extractable radioactivity decreased with time in all aerobic soils. There was a concomitant increase in "bound" radioactivity, which after one year's incubation ranged from 30-40% of the applied activity. Anaerobic soils showed greater binding than the corresponding aerobic soils. Half lives for the parent compound were 4, 6, and 8 weeks in clay, loamy sand, and clay loam respectively. Small amounts of 2-chlorobenzoic (2-chlorobenzylidine) hydrazide (13% or less of applied radioactivity), as well as N,N'bis (2-chlorobenzoyl) hydrazine, 2-chlorobenzoic acid, and 2-chlorobenzamide were found in aerobically treated soils, but did not increase over time. In sterile and anaerobic soils, the same degradates were found without significant evolution of $^{14}CO_2$ being observed.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

Results indicated half-lives of 4, 6, and 8 weeks in clay, loamy sand, and clay loam respectively. Significant quantities of $^{14}CO_2$ were formed, leaving soil bound residues. Several degradative products were detected but did not accumulate over time. Since $^{14}CO_2$ evolution was observed only in aerobic soils, microbial oxidative metabolism appears to be a highly significant pathway for the degradation of the compound.

Clofentezine is shown to be short-lived in soil.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS

This study is a carefully done, scientifically valid study, but has some deficiencies. The general conclusion of short half-life and little accumulation of degradates is supported. The major deficiency, which the applicant may be able to rectify, is lack of a pesticide treatment history for the soils. Further, the soils were taken from English sites, and were not compared with American soils of the same general type, although some soil characteristics were supplied. The moisture content of the soils was less than EPA guidelines specify, but this does not appear to invalidate the study. Acceptability of this study will depend on the further information the applicant supplies re the soil history.

10.6

A. STUDY IDENTIFICATION

The Degradation of NC 21314 in a Clay Loam and Loamy Sand Soil at 15°C. Leake, C.R. and D.J. Arnold. FBC report 25 April 1983. Study No. 42J. Report no. METAB/83/12 EPA acc. no. 262273

B. MATERIALS AND METHODS

test material
^{14}C clofentezine, sp.act. 47.4 uCi/mg, radiochemical purity > 99% by TLC
test soils

		Cottenham	Bottisham
soil type		loamy sand	clay loam
organic matter %		1.9%	14.7%
moisture holding capacity		35.3	123.0
pH		6.5	6.2
coarse sand	(2 mm- 600 um)	3.5	2.2
sand	(210-600 um)	26.3	17.8
fine sand	(105-210 um)	35.3	8.1
very fine sand	(63-105 um)	4.8	2.0
coarse silt	(20-63 um)	6.5	9.8
silt	(2-20 um)	12.4	10.8
clay	(< 2 um)	1.3	26.8

test treatment- soils prepared as described in the previous experiment (10.5) were treated with clofentezine @ 1.89 mg/kg soil and 4.33 uCi. Incubation was as described in 10.5 for aerobic samples.

Samples were taken at days 0, 7, 14, 30, and 67 post-treatment, and extracted by the first soxhlet technique described in 10.5
analyses-- as described in 10.5

C. REPORTED RESULTS

Overall recoveries of radioactivity exceeded 99% of that applied in all cases except day 67.

Extractable radioactivity decreased over time. "Bound" radioactivity increased to 20% in the clay loam and 14% in the loamy sand by day 67.

Volatile material, assumed to be $^{14}CO_2$, increased to 2.5% in clay loam and 5.5% in loamy sand by day 67.
The identifiable radioactive products in the extracts after incubation were parent compound and 2-chlorobenzoic acid. Also in the extracts were uncharacterized "polar" material (at a maximum of c. 4%), and "remainder" material (a maximum of c. 13%, and more usually c. 4-6%). CO_2, and "bound" material accounted for the radioactivity remaining. For details, see tables 10.6 and 10.6.a.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

At 15°C, clofentezine had a half life of 65 days in clay loam, and an estimated half life of 85 days in loamy sand. For details, see table 10.6.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS

This study augments the information presented in 10.5, and reinforces the conclusion that clofentezine has a relatively long half life under the given conditions. Like study 10.5, it does not provide the pesticide treatment data for the test soils, and has the other deficiencies mentioned in connection with that study. Cation exchange capacity of the soil was not specified.

10.7

A. STUDY IDENTIFICATION

Laboratory Leaching Study with NC 21314 in Three Standard Soils from West Germany. Snowdon, P.J. FBC report 25 June, 1982. Study no. 173/4/5. Report no. RESID/82/50 EPA acc. no. 262273

B. MATERIALS AND METHODS

test material-- 50% w.p. formulation (designated CR 15456)
test soils--

soil type	organic matter %	% sand	% silt	% clay	pH
loamy sand 2.1	1.8	84.7	6.2	4.2	7.9
sandy loam 2.2	4.5	72.7	14.2	5.3	6.2
sandy loam 2.3	1.9	63.4	22.1	10.8	7.3

test columns-- 350 mm l x 50 mm i.d. filled with air dried sieved soil per BBA protocol (reference: Information Leaflet no. 37 of the Biologische Bundesanstalt fur Land-und Forstwirtschaft 2nd edition February 1980), 3 for each of the 3 soils
test protocol-- filled columns were saturated with H_2O, and two for each of the soil types were treated with test material at a rate equivalent to 1 kg a.i./ha (0.5 ml of 800 ug/ml suspension). The remaining columns were untreated controls.

Each column was eluted with 400 ml H_2O @ c. 8 ml/hr for 48 hr. Eluted

water was collected in two 200 ml portions in darkened flasks, and stored for 1 day @ 1°C until analysis. The soil from the columns was then subdivided into 5 cm portions, air dried, and frozen until analyzed.

analysis

leachate-- the eluted material was concentrated by reverse-phase C_{18} Sep-pak adsorption and elution with methanol. The methanol solution was then concentrated and subjected to reverse-phase HPLC. Clofentezine was detected by UV absorption @ 268 nm. Details are referenced to another publication by the author of this study (Snowdon, P.J. FBC Report RESID/81/39 (June, 1981) "Analytical Method for Residues of NC 21314 in Soil. Reg. ref. 21314/R8=C9.)

The extract was concentrated, subjected to silica column clean-up, and analyzed by reverse-phase HPLC. Details of the procedure are referenced (Snowdon, P.J. op.cit.)

C. REPORTED RESULTS

The detection limit for clofentezine in H_2O in this procedure is 0.005 ug/ml. The recovery of 0.02 and 0.04 ug/ml spikes was 80%$\pm$ 7%. No clofentezine was detected in any of the H_2O leachates.

The chromatographic method for soil detects clofentezine at 0.006 mg/kg in a "clean" chromatogram. Since the untreated soils showed apparent residues up to 0.024 mg/kg, the effective detection limit was set at 0.03 mg/kg. Recovery of spikes ranging from 0.05-1.0 mg/kg was 87.2% $\pm$ 10.7%.

No significant residues were detected except in the top segments (0-5 cm).

Details of the results are provided in table 10.7.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

The results indicate that unaged clofentezine does not leach significantly from any of the three soils tested, since the total active ingredient was recovered from the top 5 cm of the columns.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS

This reviewer concurs with the general conclusions of the author. There are minor deficiencies which do not invalidate the study.
These are:

- the less-than-ideal recoveries from the analytical procedures, which range from only 80-87% on the average. The author has noted this and corrected the analytical results mathematically.
- the somewhat inexact determination of detection limits for the soil analysis. Since controls showed apparent residues of 0.007 to 0.024 mg/kg, the author has taken a conservative approach and chosen a limit higher than the highest value, or 0.03 mg/kg Test values are several orders of magnitude larger than this.

The test soils are from West Germany. Some soil characteristics are given in the report, although cultivation and pesticide treatment history are not given. Cation exchange capacity is not given.

The conclusion that the unaged compound has little tendency to leach is supported.

10.8

A. STUDY IDENTIFICATION

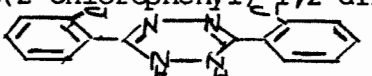
The Degradation and Leaching of NC 21314 in a Loamy Sand Soil. Leake, C.R. FBC Report 12 July 1982. Study no. 35J. Report no. METAB/82/33 EPA acc. no. 262273

B. MATERIALS AND METHODS

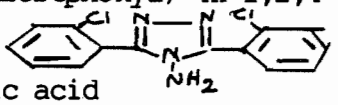
test materials

^{14}C clofentezine labelled in the tetrazine ring, sp. act. 86.4 mCi/mg 97.8% radiopure by TLC

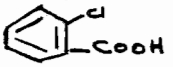
NC 22 505-- 3,6-bis(2-chlorophenyl)-1,2-dihydro-1,2,3,5-tetrazine



NC 26 414-- 3,6-bis(2-chlorophenyl)-4H-1,2,4-triazole-4-amine



NC 233-- 2-chloro-benzoic acid



test soil-- loamy sand from Cottenham, Cambridgeshire, U.K. 3.52% coarse sand (600 um), 26.31% sand (210-600 um), 35.31% fine sand (105-210 um), 4.75% very fine sand (63-105 um), coarse silt (20-63 um), silt (2-20 um), clay (2 um), 1.9% organic matter, pH 6.5

columns-- 3 columns, each made up of 7 aluminum rings held together with tape, a total height of 35 cm x 4.6 cm i.d. The packed soil was held by a nylon gauze and buchner funnel at the bottom of the column.

soil preparation-- the soil was sieved to < 2mm and incubated for as much as 1 wk @ 25°C ± 2°C. Moisture content and moisture holding capacity were checked (Hilgard cup technique, see appendix 10.8.) Three portions of 43.2 gm dry weight were weighed into individual 500 ml conical flasks.

soil treatment-- 84.5 ug of clofentezine in 0.4 ml DCM was added to each of the three soil portions, equivalent to 1.96 mg/kg soil. After evaporation of DCM the soil moisture content was adjusted to 50% of the moisture holding capacity.

sample incubation-- The three flasks were incubated as in 10.5 but with an additional trap. Aliquots of the trapping mixture were taken for LSC at days 5, 12, 26, and 31.

column preparation-- the columns were packed one segment at a time with untreated soil until 6 segments were filled. The columns were then adjusted to 50% moisture holding capacity with 0.01M $CaCl_2$. The aged and treated soil was applied to the prepared column and packed to 2 cm depth. The column was topped with glass wool to facilitate uniform distribution of "rainfall".

leaching-- columns were eluted with 0.01M $CaCl_2$ @ 25°C ± 2°C, using a flow rate of 0.4-0.5 ml/hr (the equivalent of 0.72 cm/day.) [The author notes that $CaCl_2$ was used as eluant in order to reduce clay colloid deflocculation] Leachate was collected every 24 hrs over a 45 day period. Aliquots of the leachate were analysed by LSC.

analysis-- following the last addition of eluant, the columns were drained for 48 hrs., then segmented for analysis. Each segment, the top 2 cm. (the treated soil) and 5 cm successive portions, was soxhlet extracted with 300 ml dichloromethane (DCM) for 3 hrs., and then with 300 ml methanol:H_2O (9:1) for 3 hrs. Aliquots were analyzed by LSC.

The DCM and methanol:H_2O extracts were concentrated by rotary evaporation and subjected to TLC in two solvent systems:

system 1: chloroform:methanol (49:1)
system 2: Toluene:ethanol:ethyl acetate: acetic acid (80:10:5:0.5)

soil combustion-- samples (ca. 0.3 gm) of the extracted soil were mixed with glucose and combusted. The $^{14}CO_2$ was adsorbed and counted by LSC.

C. REPORTED RESULTS

Between 4 and 10% of the applied radioactivity was recovered as $^{14}CO_2$. An estimated 24.2% additional material would also be released as $^{14}CO_2$ during the final two weeks of the study based on a decline study with this soil.

No radioactivity was found in the leachate. 30- 40% of the radioactivity was found in extracts from the top 2 cm of soil (the treated soil) and varying amounts from 0.6-14.9% in the next 5 cm. Bound residue was mostly in the top 2 cm. (14-19%) and also in the next 5 cm (1-8%). All remaining segments had less than 0.3% of the applied radioactivity. The compounds identified in the extracts were mainly parent, with trace amounts of the "reduced" compound, 2-chlorobenzoic acid, and material which remained at the origin.

Overall recovery for the three columns were 78.3, 75.7, and 72%. Correcting for the untrapped $^{14}CO_2$, a [theoretical] mean recovery of ca. 99% is obtained. For details, see table 10.8.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

This study yields results similar to those from another leaching study which indicated that leaching from a loamy sand soil is negligible. No leachable metabolites were formed.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS

The study supports the conclusion that the compound and its metabolites do not leach under the described conditions. There are two deficiencies which do not totally invalidate the study:

The author has only presented a statement summarizing the results of the product identification phase of the experiment, so actual numbers or percentages are not available for examination.

Further, the volatile products formed during the final two weeks were not trapped and quantified. Therefore, the correction for this loss of material is of unknown validity and accuracy.

The applicants should submit such additional specific data and information as they have in order to address these two deficiencies.

10.9

A. STUDY IDENTIFICATION

The Immediate Leaching of Apollo 50 SC in Three West German (Speyer) Soils. Leake, C.R. and D.J. Arnold. FBC report 29 Nov. 1985. Study no. 71J. Report no. METAB/85/32. EPA acc. no. 262273

B. MATERIALS AND METHODS (Protocols)

test materials

clofentezine-- ^{20}C labelled in the tetrazine ring, sp. act. 80 uCi/mg, 98.7% radiochemical purity by TLC

Apollo 50 SC-- formulation @ 500 gm clofentezine/l

stock solution-- 200 ul Apollo 50 SC + 2.86 mg ^{14}C labelled clofentezine in 1 ml acetone, brought to 100 ml volume with distilled H_2O

soils-- three standard soils from West Germany

		Speyer 2.1	Speyer 2.2	Speyer 2.3
soil type		sand	loamy sand	sandy loam
organic matter %		0.5%	5.0%	1.3%
CEC meq/100 gm		4.0	15.8	7.1
pH		5.5	5.0	5.5
coarse sand	(2 mm- 600 um)	5	2	4
sand	(210-600 um)	53	42	35
fine sand	(105-210 um)	25	30	23
very fine sand	(63-105 um)	5	6	5
coarse silt	(20-63 um)	6	6	15
silt	(2-20 um)	3	7	11
clay	(< 2 um)	3	7	7

columns-- glass, 350 cm x 50 mm i.d., plugged with glass wool topped with sand to fill the conical portion, filled with soil, and overlayered with glass wool to aid in even dispersion of applied water. These columns were then conditioned by washing with deionized H_2O until leachate (eluate) was collected, and drained for at least 24 hr until flow of eluate ceased.

experimental treatment--

1-- 2.0 ml stock solution was applied to each column (equivalent to 10 kg/ha). The columns were immediately eluted with 393 ml of distilled H_2O delivered at an even rate over 48 hr. Eluate was collected until the columns were completely drained.

2-- Due to the presence of clay particles in the "Soil 2.3" eluate from Experiment 1, which were not removed by centrifugation, the experiment was repeated using 0.01 M $CaCl_2$ as eluant. This successfully prevented deflocculation of the clay component.

analysis-- the leachates from the conditioning phase were used as background samples for LSC. Samples from the treatment phase were subjected to LSC, and also to partition into dichloromethane followed by TLC in two solvent systems.

C. REPORTED RESULTS

Observed amounts of radioactivity in the leachates were 2% or less. The 2 experiments with Soil 2.3 showed 2.05% of applied radioactivity in the leachate with deionized H_2O, vs 0.98% with $CaCl_2$. Soil 2.1 had 0.98%, and 2.2, 0.49%. The radioactive compound was identified as 2-chlorobenzoic acid. See table 10.9 for details.

D. STUDY AUTHOR'S CONCLUSION/QUALITY ASSURANCE MEASURES

These results, together with other experiments, indicate that the compound clofentezine is not prone to leaching from the soils tested.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

The author's conclusion, that clofentezine does not leach under described conditions, is supported. It should be noted that the material was not aged before application of leachant. The soils are not from the U.S., but the characteristics are specified.

10.10

A. STUDY IDENTIFICATION

The Leaching of NC 21314 in Four Soil Types Using Soil TLC. Leake,C.R. and D. Lines. FBC report 18 Jan, 1982. Study no. J7. Report no. METAB/81/10 EPA acc. no. 262273

B. MATERIALS AND METHODS (Protocols)

test materials

clofentezine-^{14}C labelled in the tetrazine ring, sp. act. 86.4 uCi/mg, radiopurity >95%, stock solution in acetone 12.01 ug and 8.13×10^{-3} uCi/100 ul

atrazine-^{14}C labelled in the triazine ring, sp. act. 19.1 uCi/mg, radiopurity >86%, stock solution in acetone 1.75 ug and 7.33x 10^{-2} mCi/100 ul

2,4-D-^{14}C labelled in the alkyl carbon of the oxyacetic acid sidechain, sp. act. 242 mCi/mg, radiopurity >98%, stock solution in acetone 21.23 ug and 0.021 uCi/100 ul

paraquat-^{14}C labelled in the dipyridylium rings, sp. act. 167 uCi/mg, radiopurity >95%, stock solution in acetone/H_2O (95/5) 0.2538 mg and 6.33×10^{-3} mCi/300 ul

soils

soil type	Organic matter	pH	% sand coarse/fine		% silt coarse/fine		%clay
sand	0.8%	7.2	51.44	31.93	0.05	6.65	1.25
sandy loam	5.9%	5.8	6.85	52.22	9.90	5.05	12.96
silt loam	3.8%	7.5	4.24	10.16	17.72	39.00	23.08
clay	4.5%	6.6	30.80	6.58	2.80	3.15	49.23

soil thin-layer preparation

The clay and silt loam soils were sieved to < 250 um and the sandy loam and sand were sieved to < 500 um. A slurry of each soil was

prepared in distilled H_2O and spread onto TLC plates (@ 500 um for clay and silt loam, and 1000 um for sandy loam and sand.)

TLC analysis

Compounds were applied along a baseline 1.5 cm from the bottom of the plate, and eluted using 0.01 M $CaCl_2$ for a distance of ca. 10 cm.

The radioactive areas were located by autoradiography.

C. REPORTED RESULTS

The three reference compounds showed widely variable but measurable rf values (table 10.10) between soils and between compounds, but NC 21314 showed no mobility in any of the test soils.

D. STUDY AUTHOR'S CONCLUSION/QUALITY ASSURANCE MEASURES

NC 21314 can be classified as immobile in all soils tested. The work done in this study is in general agreement with the work of Helling as described in the references given in attachment 10.10.a. Experiments of this type are useful for qualitative comparisons, but it should be noted that the behavior of pesticides on soils in natural conditions is subject to many other variables than this experiment addressed.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

The conclusions of the author are supported by the data presented. This, together with the other soil mobility studies, demonstrates that this pesticide is not readily leached from soils.

10.11

A. STUDY IDENTIFICATION

Residue Decline Study in Soil Following Application of NC 21314 to Bare Plots in Texas, 1980. Snowdon, P.J. FBC report 22 March 1982. Study no. 173/4/2. Report no. Resid/82/19. EPA acc. no. 262273

The manner of application of the pesticide was not in accordance with any proposed label directions (i.e. application to bare ground). Therefore the data developed are not directly applicable to environmental fate concerns. The author reports a general lack of significant residues below the top 7.5 cm of soil, and also reports projected half lives of 34-83 days (based on two replicates each at two application rates.)

10.12

A. STUDY IDENTIFICATION

Decline of Clofentezine Residues in Soil Following Orchard Treatment with the 50 SC Formulation in New York, USA, 1984. Snowdon, P.J. FBC report 26 Nov. 1985. Study no. 073/04/008. Report no. RESID/85/64. EPA acc. no. 262273

B. MATERIALS AND METHODS (Protocols)

test material— 2% a.i. (as a suspension of 50% concentrate clofentezine, company code CR 16244)
test treatment— test material applied at a rate of 1120 l/ha to a McIntosh apple orchard, 3 plots of 9 trees each, once prebloom and once with the fruit at 2.5-4 cm size
test sampling-- 2 cores taken from the dripline around each tree and cores midway between trees at 1, 3, 7, 14, 28, and 63 days following the first treatment, and at 1, 3, 7, 14, 35, 67, 95, 123, and 156 days following the second treatment. These cores were subdivided into 0-7.5 cm and 7.5-15.0 cm. The corresponding cores (equivalent treatment and depth) were composited and stored frozen. The samples were air-dried, sieved and ground, and frozen again until analysis.
analysis-- the soil was soxhlet extracted with acetone. The acetone was diluted with H_2O, partitioned into hexane, subjected to a silica column clean-up, and finally analyzed by HPLC. Clofentezine was detected by light absorption at 535 nm.
weather conditions— see confidential appendix, table 10.12

C. REPORTED RESULTS

Recoveries of samples spiked at levels from 0.05-0.50 mg/kg ranged from 75%-125%, with 10/13 greater than 90%. The overall mean recovery of 99% from these samples was used as a correction factor for the experimental results. The limit of detection was determined to be 0.05 mg/kg, based on results from untreated soil. In the treated samples, 9/15 of the mean values for 0-7.5 cm segments were < 0.05 mg/kg, and the rest ranged from 0.05-0.07. The highest individual value was 0.10 mg/kg, from a sample taken the day after the second application. No 7.5-15.0 cm segment analyzed above background levels, based on samples taken two weeks following each of the two treatments. See table 10.12.a for details.

D. STUDY AUTHOR'S CONCLUSION/QUALITY ASSURANCE MEASURES

These results are in agreement with the leaching studies, indicating little or no leaching. Further, the study indicates soil levels which are at or very close to the detection limit following treatment of orchard trees. This confirms that little soil residue can be expected from this method of application.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

The conclusions of the author appear to be supported. However, it is not stated whether "spiked" samples used to determine recovery were handled in the same manner as the experimental samples, i.e. aged, frozen, etc. The applicant should supply this information. Fresh spiked samples would show the maximum recovery, while aged frozen samples might show much less due to decomposition and/or adsorption of the compound, especially since this compound is highly adsorbed to soil.

10.13

A. STUDY IDENTIFICATION

Residue Decline Study in Soil Following Annual Single and Double Applications of NC 21314 (50W) at Shelford, U.K., Commencing 1981: Decline of Residues following 1981 Applications. Snowdon, P.J. FBC report 6 Oct., 1983. Study no. 173/4/6. Report no. RESID/83/70. EPA acc. no. 262273

The test material was applied to bare ground (sandy loam soil), which is not a recommended application method for actual use, therefore a full review of this study will not be done. The 1983 study (q.v.) will be reviewed in full.

Following a single application of test material at 1 kg/ha, the residue found at day 0 was 1.07 mg/kg, which declined to 0.023 mg/kg by day 149. The author projects a half-life in the top 0-7.5 cm segment of 54.0 $\pm$ 13.6 days based on these data.

10.14

A. STUDY IDENTIFICATION

Residue Decline Study in Soil Following Annual Single and Double Applications of NC 21314 (50W) at Shelford, U.K., Commencing 1981: Decline of Residues following 1982 Applications. Snowdon, P.J. FBC report 10 June, 1983. Study no. 173/4/6. Report no. RESID/83/31. EPA acc. no. 262273

This study is a continuation of the previous one (10.13), and has the same objections. Projected half-life in the 0-0.75 cm segments was 32.4 $\pm$ 3.6 days.

10.15

A. STUDY IDENTIFICATION

Residue Decline Study in Soil Following Annual Single and Double Applications of NC 21314 (50W) at Shelford, U.K., Commencing 1981: Decline of Residues following 1983 Applications. Snowdon, P.J. FBC report 16 July, 1984. Study no. 073/04/006. Report no. RESID/84/45. EPA acc. no. 262273

B. MATERIALS AND METHODS

test material-- clofentezine formulated as 50 WP
test treatment-- applied at a rate of 1 kg a.i./ha during the 1983 growing season to 2x10 m plots of bare soil in Shelford, Cambs., England. Two plots were untreated controls, two were treated once, and two twice. The same plots had been similarly treated during 1981 and 1982. Single application was made on May 4, 1983. Doubly treated plots were sprayed on May 4 and June 15, 1983.
sampling-- a minimum of 15 cores from each plot, taken with a 3.8 cm i.d. auger, to 7.5 cm depth. Cores from each plot were combined. frozen, air dried, sieved, ground, and refrozen until analysis.

sampling intervals-- days since last application
single spray— days 0, 7, 13, 30, 56, 71, 103, 140, 168, 212, 323
double spray-- days 0, 7, 14, 29, 61, 108, 136, 180, 291
residue analysis-- the soil was extracted by reflux with a dichloromethane: methanol mixture. The extract was concentrated, cleaned up with a silica column, and subjected to HPLC. Clofentezine was located by visible light absorption @ 535 nm. Since other work indicated that clofentezine is resistant to metabolism and other degradative processes and that the other important metabolite is CO_2, only the parent compound was sought in these analyses.

C. REPORTED RESULTS

The analytical detection limit was determined to be 0.01 mg/kg. Results from "spiked" samples indicated 97.5% $\pm$ 5.1% recovery, and this figure was used to correct experimental results. For the single treatment plots, pretreatment level was 0.05 mg/kg. The "day 0" level was 1.01, declining to 0.08 after 323 days. For the doubly treated plots, the level before the second treatment was 0.12 mg/kg. At "day 0" it was 1.90 mg/kg, declining to 0.17 mg/kg after 191 days. See details in table 10.15.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

The projected half-life for clofentezine was 51.9 $\pm$ 5.0 days in this experiment. The data from the last two samples [from the single treatment plots] appear to indicate a second mechanism taking over during this period, which resulted in slower degradation. These data were not included in deriving the half-life. These data demonstrate that clofentezine residues do not accumulate in the soil in these conditions. Since the act of sampling caused movement of the compound into deeper layers of soil, later samples were taken only from the top 7.5 cm.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

Although the explanation offered by the author (re the aberrent data from day 212 of the single treatment) is certainly possible, no other data point is obviously out of line with the others. A far simpler explanation is that this sample was somehow flawed, not well mixed or otherwise untypical.

As mentioned in connection with a previous study, sample handling may not have been equivalent to that of "spiked" controls. This objection may be answered by providing a statement detailing this information.

These data, as well as data from the previous two years, support the conclusion that clofentezine does not accumulate in soil. Other evidence has been presented to demonstrate the lack of significant amounts of other extractable metabolites.

10.16

A. STUDY IDENTIFICATION

Summary of Residue Decline Studies in Soil following Annual Single and Double Applications of Clofentezine (50W) at Shelford, UK. between 1980 and 1983. Snowdon, P.J. FBC report 12 Sept. 1985. Study no. 073/04/006.

Report no. RESID/85/69. EPA acc. no. 262273

A full review of this study will not be done, since it merely summarizes results of the three previous studies.

10.17

A. STUDY IDENTIFICATION

Determination of the Accumulation of NC 21314 in Bluegill Sunfish (Lepomis macrochirus) using a Dynamic Test System. Hill, P.W. J.E. Caunter, and E. Gillings. ICI and FBC report Dec. 1982. Report no. METAB/92/50

B. MATERIALS AND METHODS (Protocols)

test material

^{14}C clofentezine-- sp. act. 87.15 $\pm$ 5.79 uCi/mg, radiopurity 98% by HPLC

technical clofentezine-- 99.8% pure

stock solution-- ^{14}C clofentezine + technical at a final sp. act. of 16.22×10^6 Bq/gm

test treatment-- clofentezine was adsorbed onto pumice, packed into columns and delivered by a continuous flow system (250 ml/minute) to the fish tank at an analyzed rate of 0.018 mg/l (range 0.0145-0.0244 mg/l), or approximately 2/3 saturation. Before treatment of the fish, the tank was determined by analysis to have reached clofentezine equilibrium.

tank conditions-- temp. 22 $\pm$ 1°C, oxygenated with filtered compressed air passed through an air diffuser. The size was 880 x 370 x 270 mm, total volume ca. 68 l.

exposure-- 100 bluegill sunfish weighing an average 1.23 gm (range 0.75-1.95) and an average 37 mm length (range 32-44) were exposed for 28 days in the conditions described. A parallel group of 100 (average weight 1.18 gm, range 0.42-2.90; average length 35.7 mm, range 29-49) was maintained under similar conditions without clofentezine exposure. The fish were fed a commercial food at the rate of 1% of the average body weight of the fish each day. Following exposure, the experimental fish were subjected to depuration in fresh water, the other conditions remaining the same.

analyses

clofentezine in the fish-tank water-- analyzed by LSC and HPLC

radioactivity in fish-- total ^{14}C residues in whole fish, determined by combustion and LSC of the trapped $^{14}CO_2$.

water quality-- dissolved oxygen, pH, temperature, and flow rates were measured all through the study together with regular checks of hardness, alkalinity, conductivity, and free and residual chlorine. Alpha-BHC, gamma-BHC, dieldrin, o-p-DDT, p-p-DDT, and Arochlor 1254, un-ionized ammonia, total organic carbon, suspended solids, cobalt, copper, iron, lead, manganese, nickel, and zinc were also measured during the study.

C. REPORTED RESULTS

Levels of clofentezine in the water had a range of 0.0145-0.0244 mg/l (mean of 0.018 $\pm$ 0.003 mg/l) with good agreement between LSC and HPLC analyses. Whole fish had a level of 7.77 mg/kg (wet weight) after 28 days exposure. This represents a bioconcentration factor (BCF) of 7.77 $\div$ 0.018

or 430. Radioactive residues were 88% eliminated by three days of depuration, and 94% eliminated within seven days. Details are in table 10.17.

2 treated fish (2%) died during the exposure phase, and none during the depuration phase. No control fish died during either phase. Weights and weight gains at the end of the experiment were comparable in the two groups.

D. STUDY AUTHOR'S CONCLUSION/QUALITY ASSURANCE MEASURES

Due to the low solubility of the compound, the maximum level which could be maintained by the use of saturation columns (ca. 0.018 mg/l) was tested. Agreement between the two analytical methods indicates that clofentezine does not degrade in this test system. Further, the LC_{50} is obviously higher than the concentration used in this experiment.

The results show that the fish reach a steady state within three days of exposure, and rapidly clear the material when exposure is discontinued.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

The conclusions seem to be supported by the data. However, there are serious deficiencies in the study. These deficiencies may be remediable:

first-- the site of labelling is not specified. We must have this information to evaluate the conclusions reached.

second-- the analyses for viscera and edible portions of the fish were not given. If this analysis was done, we need these data. If it was not done, the study is unacceptable.

third-- the tissue must be analyzed for parent and metabolites. No results were reported. If the analyses were done, we need the data. If they were not done, the study is unacceptable.

Further, the feeding rate given the fish should be clarified-- the figure given is probably a per-fish rate, but this needs to be specified. The concentrations in the individual fish during the accumulation phase are much more variable than corresponding concentrations after exposure is discontinued, as is to be expected.

11. COMPLETION OF ONE-LINER: not initiated at this time

12. CBI APPENDIX: (if applicable): included