Data Evaluation Record on the hydrolysis of clofentezine

PMRA Submission Number {......} EPA MRID Number 47192107

Data Requirement: PMRA Data Code:
EPA DP Barcode: D342560
OECD Data Point:
EPA Guideline: 161-1

Test material:

Common name:	Clofentezine.
Chemical name:	
IUPAC name:	3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS name:	3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS No.:	74115-24-5.
Synonyms:	NC 21314, NC 21 314, AE B084866.
Smiles string:	Clc1ccccc1c2nnc(c3ccccc3Cl)nn2 (EPI Suite, v3.12 SMILES).

Primary Reviewer: Leanne Ganser
Cambridge Environmental

Signature:
Date: 12/19/07

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Date: 02/20/08

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 125501

CITATION: van der Gaauw, A. 2001. [^{14}C]-Clofentezine: Hydrolysis at three different pH values. Unpublished study performed by RCC Ltd., Itingen, Switzerland; and sponsored and submitted by Irvita Plant Protection N.V., Curacao, the Netherlands Antilles. RCC Study No.: 793124. Sponsor Project No.: R-13318. Experiment started February 14, 2001 and completed May 22, 2001 (p.10). Final report issued November 14, 2001.

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EXECUTIVE SUMMARY

The hydrolysis of [1,2,4,5-tetrazine-^{14}C]-labeled 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine (clofentezine; radiochemical purity ≥99.9%), at 2.1 µg a.i./L, was studied in the dark at 49°C in sterile, aqueous pH 4 (0.01M citrate), pH 7 (0.01M imidazole), and pH 9 (0.01M borate) buffer solutions for 5 days, at 35°C in the pH 7 buffer solution for 14 days, and at 25°C in the pH 7 buffer solution for 21 days. The experiment was conducted in accordance with OECD Guideline for Testing Chemicals 111 "Hydrolysis as a function of pH" and Directive 92/69/EEC part C.7 and in compliance with the Swiss Ordinance relating to GLP. The test system consisted of brown glass vessels (100 mL) filled with treated, degassed buffer solution (100 mL) sealed with lined screw caps and incubated with constant agitation in the dark. Volatiles were not collected. Duplicate samples were collected for analysis at 0, 0.1, 1, and 5 days posttreatment for the 49°C experiment, at 0, 1, 2, 3, 4, 7, 8, 10, and 14 days for the 35°C/pH 7 experiment and at 0, 1, 2, 3, 4, 7, 10, 14, and 21 days posttreatment for the 25°C/pH 7 experiment. The samples were partitioned twice with dichloromethane, concentrated by rotary evaporation and dissolved in acetonitrile. Samples were analyzed by LSC and HPLC. [^{14}C]Clofentezine was identified by co-chromatography with an unlabeled reference standard of clofentezine (purity 99.7%). Identifications were confirmed with TLC analysis.

The temperature of the buffer solutions was reported as 49.1 ± 0.2°C, 35.5 ± 0.0°C and 25.01 ± 0.1°C. Supporting data were not provided. The pHs of the untreated buffer solutions were 4.00-4.13, 6.90-7.20, and 8.99-9.06. The buffer solution remained sterile throughout the study.

In the **<u>49°C/pH 4, 7 and 9 experiment</u>**, overall recoveries were 97.7 ± 2.5% of the applied in the pH 4 buffer, 100.5 ± 1.3% in the pH 7 buffer, and 97.3 ± 4.9% in the pH 9 buffer. Recoveries were low in both the pH 4 and pH 9 buffers at 5 days posttreatment (95.0 and 89.4% of the applied, respectively). In the **pH 4 buffer**, [^{14}C]clofentezine was relatively stable, decreasing from 99.9% of the applied at time 0 to 95.0% at 5 days posttreatment. No major or minor transformation products were isolated. In the **pH 7 and 9 buffers**, [^{14}C]clofentezine hydrolyzed rapidly, forming three transformation products:

- 2-chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1)
- 2-chlorobenzonitrile (M2) and
- 2-chlorobenzamide (M5).

In the **pH 7 buffer**, [^{14}C]clofentezine decreased from 99.5% of the applied at time 0 to 83.3% at 0.1 day posttreatment and 2.8% at 5 days. 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) averaged a maximum of 91.8% of the applied at 1 day posttreatment, decreasing to 81.6% at 5 days. 2-Chlorobenzonitrile (M2) averaged a maximum of 12.3% of the applied at 5 days posttreatment.

In the **pH 9 buffer**, [^{14}C]clofentezine decreased from 93.8% of the applied at time 0 to ≤1.7% at 0.1 and 5 days posttreatment. 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) averaged a maximum of 93.7% of the applied at 1 day posttreatment, decreasing to 25.1% at 5 days. 2-Chlorobenzonitrile (M2) averaged a maximum of 62.6% of the applied at 5 days posttreatment. The study author reported that 2-chlorobenzamide (M5) was recovered in the M2 fraction, but did not provide any quantitative data.

In the **25°C/pH 7 experiment**, overall recoveries were 101.6 ± 1.2% of the applied (range 99.8-104.3%). There was no pattern of loss of material over time.

[^{14}C]Clofentezine decreased rapidly from an average 93.5% of the applied at time 0 to 55.4% at 1 day to 23.6% at 2 days and was last detected at 6.2% at 4 days posttreatment. Based on first order linear regression analysis (Excel 2003), clofentezine dissipated from the pH 7 buffer solution with a calculated half-life of 1.0 days. The observed DT_{50} was *ca.* 1 day. Using nonlinear regression analysis, the calculated half-life is 1.1 days for clofentezine.

Two major transformation products were isolated. 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) averaged a maximum of 42.2% of the applied at 1 day posttreatment and was 0.0% and 5.1% at 14 days. 2-Chlorobenzonitrile (M2) plus M4, which was a solvent artifact of M2, totaled 95.8% of the applied at 21 days posttreatment. Two minor transformation products were isolated. 2-Chlorobenzamide (M5), averaged a maximum of 8.5% of the applied. Unidentified M3 averaged a maximum of 4.8% of the applied. Volatiles were not collected.

In the **35°C/pH 7 experiment**, overall recoveries were 101.2 ± 1.7% of the applied (range 98.7-104.4%). There was no pattern of loss of material over time. [^{14}C]Clofentezine decreased from 93.5% of the applied at time 0 to 17.8% at 1 day posttreatment and was not detected after 1 day. A half-life was not calculated due to insufficient sampling intervals. The study author estimated graphically a half-life of 0.6 days. Maximum concentrations of M1, M2, M3, M4 and M5 were 83.9%, 57.2%, 3.7%, 42.4% and 9.6%, respectively.

A transformation pathway was provided by the study author. In that proposed pathway, clofentezine hydrolyzes to 2-chlorobenzoic acid (2-chlorobenzylidene)hydrazide, which is then rapidly hydrolyzed to at least two compounds, identified as 2-chlorobenzonitrile and 2-chlorobenzamide, which are stable to hydrolysis.

RESULTS SYNOPSIS:

pH	Half life (days)	Transformation products
At 25°C		
7	**1.0**	2-Chlorobenzamide.
At 35°C		
pH 7	Not Calculated	2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide. 2-Chlorobenzonitrile. 2-Chlorobenzamide.
At 49°C		
	DT_{50} (days)	
4	>>5 days (<5% decrease over 5 days)	None.
7	<1 day	2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide. 2-Chlorobenzonitrile. 2-Chlorobenzamide.
9	<0.1 day	2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide. 2-Chlorobenzonitrile. 2-Chlorobenzamide.

Study Acceptability: This study is classified as **acceptable**. No significant deviations from good scientific practices were noted. The 25°C experiment was conducted only at pH 7, although significant degradation had occurred in the pH 9/49°C experiment. The pH 4/49°C experiment found clofentezine to be stable and so a 25°C pH 4 test was not required.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with OECD Guideline for Testing Chemicals 111 "Hydrolysis as a function of pH" (1981) and Directive 92/69/EEC part C.7 (1992) and under consideration of EPA OPPTS 835.2110 (p.11). The following significant deviation from the objectives of Subdivision N guidelines was noted:

> The 25°C experiment was conducted only at pH 7, although significant degradation had occurred in the pH 9/49°C experiment.

COMPLIANCE: This study was conducted in compliance with the Swiss Ordinance relating to GLP (RS 813.016.5; 2000; p.3). Signed and dated Data Confidentiality, GLP and Quality Assurance statements provided (pp.2-3, 3a, 5). A Certificate of Authenticity was not provided; a Signatures pages was provided (p.4).

A. MATERIALS:

1. Test Material [1,2,4,5-Tetrazine-^{14}C]clofentezine (p.14).

Chemical Structure: See DER Attachment 1.

Description: Magenta solid, technical grade (p. 7; Appendix 1, p.54).

Purities:
Radiochemical purity: ≥99.9% (p.14).
Batch No.: GAR 1957/5.
RCC No.: 111267/A.
Analytical purity: Not reported.
Specific activity: 1568.7 MBq/mmol; 42.4 mCi/mmol; 139.2 µCi/mg; 5.15 MBq/mg.
Location of the radiolabel: Uniformly in the 1, 2, 4, 5-tetrazine ring.

Storage conditions of test chemicals: The test substance was stored at *ca.* -20°C in the dark (p.14). An unlabeled standard of clofentezine was stored at room temperature. Other reference standards were stored below 30°C (p.15).

Physico-chemical properties of clofentezine:

Parameter		Value	Comment
Molecular weight		303.15 g/mol.	
Molecular formula		$C_{14}H_8Cl_2N_4$.	
Water Solubility		2.5 µg/L.	At 22°C, pH 5.
Solubility in solvents	Dichloromethane Acetone Hexane Ethanol	37 g/L. 9.3 g/L. 1 g/L. 0.5 g/L.	At 25°C.
Vapor Pressure/Volatility		Not reported.	
UV Absorption		Not reported.	
pKa		Not reported.	
K_{ow}/log K_{ow}		Not reported.	
Stability of compound at room temperature, if provided		Not reported.	

Data obtained from pp.13-14; Appendix 1, pp.53-54 of the study report.

2. Buffer Solution: Buffer solutions were prepared with deionized water purified using a ELGA water purifier as follows and pH adjusted using 0.1M NaOH or HCl (p.17).

Table 1: Description of buffer solutions.

pH	Type and molarity of buffer	Composition
4	≤0.01M Citrate	0.357 g Monohydrate citric acid, 0.078 g sodium chloride and 0.083 g sodium hydroxide were combined and diluted to 1 L with water.
7	≤0.01M Imidazole	1.36 g Imidazole was diluted in 960 mL water and adjusted to pH 7.0 with *ca.* 10 mL of 1M HCl. The solution was diluted to 1 L with water.
9	≤0.01M Borate	0.144 g Potassium dihydrogen phosphate and 1.020 g di-sodium tetraborate were combined and diluted to 2 L with water.

Data obtained from p.17 of the study report.

B. EXPERIMENTAL CONDITIONS

1. Preliminary Study: To determine hydrolysis of the test substance at 49°C, [^{14}C]clofentezine, at 2.1 µg a.i./L, was maintained in the dark in pH 4, 7 and 9 buffer solutions for 5 days (pp.18, 20). Duplicate samples were collected at 0, 0.1 (2.4 hours), 1 and 5 days. Duplicate aliquots (5 mL) were analyzed by LSC. The samples were concentrated as described in the definitive experiment, prior to HPLC and TLC analysis.

Overall recoveries were 97.7 ± 2.5% of the applied in the pH 4 buffer, 100.5 ± 1.3% in the pH 7 buffer, and 97.3 ± 4.9% in the pH 9 buffer (Table 2, p.32). Recoveries were low in both the pH 4 and pH 9 buffers at 5 days posttreatment (95.0 and 89.4% of the applied, respectively).

In the **pH 4 buffer**, [^{14}C]clofentezine was relatively stable, decreasing from 99.9% of the applied at time 0 to 95.0% at 5 days posttreatment (Table 3, p.33). No major or minor transformation products were isolated.

In the pH 7 and 9 buffers, [^{14}C]clofentezine hydrolyzed rapidly, forming three transformation products:

- 2-chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1)
- 2-chlorobenzonitrile (M2) and
- 2-chlorobenzamide (M5).

In the **pH 7 buffer**, [^{14}C]clofentezine decreased from 99.5% of the applied at time 0 to 83.3% at 0.1 day posttreatment and 2.8% at 5 days (Table 3, p.33). 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) averaged a maximum of 91.8% of the applied at 1 day posttreatment, decreasing to 81.6% at 5 days. 2-Chlorobenzonitrile (M2) averaged a maximum of 12.3% of the applied at 5 days posttreatment.

In the **pH 9 buffer**, [^{14}C]clofentezine decreased from 93.8% of the applied at time 0 to ≤1.7% at 0.1 and 5 days posttreatment (Table 3, p.33). 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) averaged a maximum of 93.7% of the applied at 1 day posttreatment, decreasing to 25.1% at 5 days. 2-Chlorobenzonitrile (M2) averaged a maximum of 62.6% of the applied at 5 days posttreatment.

The study author reported that 2-chlorobenzamide (M5) was recovered in the M2 fraction, but did not specify whether this was the case at both pHs and did not provide any quantitative data.

2. Experimental conditions

Table 2: Experimental parameters

Parameters		**Details**
Duration of study		21 days.
Test concentrations (mg a.i./L) Nominal: Measured:		 0.0021 mg a.i./L. 0.002071 mg a.i./L.
No. of replications		Duplicate samples of each treatment were collected at each interval.
Preparation of test medium	Volume used/treatment	160 µL/ 100 mL buffer.
	Method of sterilization	Buffer solutions were autoclaved for *ca.* 30 minutes at 121°C. Vial caps were rinsed with ethanol/water (70:30, v:v).
	Co-solvent	Acetonitrile, 0.2%.
Test apparatus (type/material/volume)		Treated buffer solutions (100 mL) were placed in brown glass vessels (100 mL) sealed with lined screw caps and incubated with constant agitation in the dark.
Details of traps for volatile, if any		Volatiles were not collected.
If no traps were used, is the test system closed/open?		Closed.
Is there any indication of the test material adsorbing to the walls of the test apparatus?		No.
Experimental conditions Temperature (°C): Lighting: pH ranges:		 25°C. Dark. 6.90-7.19.
Other details, if any		The treated buffer solutions were degassed by ultrasonicating.

Data were obtained from pp.17-20; Table 1, p.31 of the study report.

3. Supplementary Experiments: The experiment was also performed at 35°C using the conditions described in the definitive experiment (pp.19-20). Duplicate samples were collected at 0, 1, 2, 3, 4, 7, 8, 10 and 14 days posttreatment. Duplicate aliquots (5 mL) were analyzed by LSC (pp.20-21). The samples were concentrated as described in the definitive experiment, prior to HPLC and TLC analysis.

4. Sampling:

Table 3: Sampling details.

Criteria	Details
Sampling intervals	0, 1, 2, 3, 4, 7, 10, 14 and 21 days.
Sampling method	Duplicate vessels were collected at each interval.
Method of collection of CO_2 and organic volatile compounds	Volatiles were not collected.
Sampling intervals/times for: pH measurement: Sterility check:	 At each sampling interval. At study initiation and termination.
Sample storage before analysis	Not reported.
Other observation, if any:	None.

Data were obtained from pp.20, 24; Table 4, p.34 of the study report.

C. ANALYTICAL METHODS

Extraction/clean up/concentration methods: The samples were partitioned twice by shaking with dichloromethane (2 x 30 mL; p.20). The organic phases were pooled and evaporated by rotary evaporation at 30°C. The residue was dissolved in acetonitrile (1 mL) and analyzed by LSC, HPLC and TLC.

Volatile residue determination: Volatiles were not collected.

Total ^{14}C measurement: Aliquots (≤5 mL) of each buffer solution were analyzed for total [^{14}C]residues using LSC (pp.20-21).

Derivatization method, if used: A derivatization method was not employed.

Identification and quantification of parent compound: The concentrated samples were analyzed directly by HPLC under the following conditions (pp.20-22): LiChrospher 100 C-18 pre-column (4 x 4 mm, 5 µm), Luna 3 µL C8 column (150 x 3 mm, 5 µm), a gradient mobile phase of (A) water acidified to pH 2 with phosphoric acid and (B) acetonitrile [percent A:B (v:v) at 0 minutes, 80:20; 20 minutes, 20:80; 25 minutes, 10:90; 30 minutes, 20:80; 30.1-35 minutes, 80:20], flow rate 0.43 mL/minutes, with UV (268 nm) and radioflow detection. [^{14}C]Clofentezine was identified by co-chromatography with an unlabeled reference standard of clofentezine (purity 99.7%, p. 16; Figure 9, p.45).

HPLC results were confirmed using one-dimensional TLC on silica gel 60 F_{254} plates with the following solvent systems (pp.22-23):

Ethyl acetate (SS 1);
Toluene:ethyl acetate:25% ammonium hydroxide (50:50:0.2, v:v:v; SS 2);
Acetone:ethyl acetate:water:acetic acid (45:45:5:5, v:v:v:v; SS 3);
Ethyl acetate:acetone:water:acetic acid (89:10:0.5:0.5, v:v:v:v; SS 4);

Ethyl acetate:acetone:water:acetic acid (88:10:1:1, v:v:v:v; SS 5);
Ethyl acetate:acetone:water:acetic acid (94:5:0.5:0.5, v:v:v:v; SS 6); and
Hexane (SS 7).

TLC was also performed on RP-18 plates using acetonitrile:water acidified to pH 2 with H_3PO_4 (50:50, v:v; SS 8; pp.22-23). Methanol was used to desorb radioactivity at the origin of select TLC plates. Plates were developed without chamber saturation. Radioactivity was detected with an Automatic TLC-Linear Analyzer and by phosphor imaging. Unlabeled reference standards were visualized by quenching of UV-light (254 nm) or in an iodine chamber. Clofentezine was identified by co-chromatography with an unlabeled reference standard (purity 99.7%, p.16; Figures 11-12, pp.47-48).

Identification and quantification of transformation products: Transformation products were identified and quantified using HPLC and TLC as for the parent compound (pp.20-23). Compounds were identified by comparison with the following unlabeled reference standards (p.16):

Applicants Code Name	Chemical Name	Purity (%)	Batch No.
R1	2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide.	97	R000164
R2	2-Chlorobenzonitrile.	99.9	R000834
R3	2-Chlorobenozic acid.	98.9	R000833
R4	2-Chlorobenzaldehyde.	98	R000832
R5	2-Chlorobenzamide.	99.9	R001613

Detection limits (LOD, LOQ) for the parent compound: For LSC analysis, the limit of detection was 1.25% of the applied and the limit of quantification was 1.88% of the applied (p.25). For HPLC analysis, the limit of quantification was 3.7% of the applied (p.26). The limit of detection for HPLC was not reported.

Detection limits (LOD, LOQ) for the transformation products: The limits of detection and quantification were the same as for the parent compound (pp.25-26).

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: During the study, the temperature of the buffer solution was maintained at 25.01 ± 0.1°C for 21 days; supporting data were not provided (p.28). The pH of the treated buffer solution was 6.90-7.19 (Table 1, p.31). The buffer solution remained sterile throughout the study (Table 4, p.34).

B. MASS BALANCE: Overall recoveries were 101.6 ± 1.2% of the applied (range 99.8-104.3%) in pH 7 buffer (Tables 5, p.35; DER Attachment 2). There was no pattern of loss of material over time from the buffer solution.

Table 4: Hydrolysis of clofentezine, expressed as percentage of the applied (mean ± sd, n = 2), at pH 7 at 25°C.

Compound	**Sampling times (days)**								
	0	1	2	3	4	7	10	14	21
Clofentezine	93.5 ± 4.0	55.4 ± 2.8	23.6 ± 0.1	16.0 ± 0.1	6.2 ± 0.7	--	--	--	--
2-Chlorbenzoic acid (2-chlorobenzylidene) hydrazide (M1)	3.2 ± 1.2	42.2 ± 0.6	39.0 ± 0.3	9.3 ± 0.1	9.6 ± 5.4	5.6 ± 0.6	8.2 ± 2.0	--, 5.1	--
2-Chlorobenzonitrile (M2)	--	2.3 ± 1.4	17.0 ± 0.1	39.0 ± 0.4	47.6 ± 1.8	49.7 ± 3.0	53.9 ± 0.6	48.9 ± 3.1	55.6 ± 0.1
Unknown (M3)	3.4 ± 2.5	1.6 ± 0.6	--	4.7 ± 0.1	2.2 ± 0.6	--	3.2, --	4.8 ± 1.3	--
M4[1]	--	--, 3.2	20.0 ± 2.4	25.8 ± 0.3	29.2 ± 3.4	38.8 ± 4.6	31.3 ± 0.7	37.4 ± 0.4	40.2 ± 0.8
2-Chlorobenzamide (M5)	--	--	3.5 ± 0.0	6.1 ± 0.1	6.8 ± 0.1	6.6 ± 0.0	7.0 ± 1.2	8.5 ± 0.3	7.2 ± 0.6
CO_2	Volatiles not collected.								
Volatile organics	Volatiles not collected.								
Total Recovery	100.0 ± 0.3	103.0 ± 1.8	101.3 ± 0.6	100.8 ± 1.1	101.5 ± 0.0	100.6 ± 1.0	102.0 ± 0.9	102.0 ± 0.8	102.9 ± 0.2

Means and standard deviations calculated using data obtained from Table 5, p.35 of the study report (DER Attachment 2).

1 Corresponds to 2-chlorobenzonitrile due to HPLC artifact.

--: Not detected or below limit of quantitation.

C. TRANSFORMATION OF PARENT COMPOUND: At pH 7 and 25°C, [^{14}C]Clofentezine decreased rapidly from an average 93.5% of the applied at time 0 to 55.4% at 1 day to 23.6% at 2 days and was last detected at 6.2% at 4 days posttreatment (Table 5, p.35; DER Attachment 2).

HALF-LIVES/DT$_{50}$/DT$_{90}$: Based on first order linear regression analysis (Excel 2003), clofentezine dissipated from the pH 7 buffer solution with a reviewer-calculated half-life of 1.0 days (DER Attachment 2). The observed DT$_{50}$ was *ca.* 1 day (Table 5, p.35). Using nonlinear regression analysis, the study author calculated a half-life of 1.1 days for clofentezine (pp.26-27; Figure 14, p.50).

Half-lives/DT$_{50}$/DT$_{90}$

pH	First order linear[1]			DT50[2] (days)	DT90[2] (days)
	Half-life (days)	Regression equation	r^2		
7	1.0	y = -0.6672x + 4.5954	0.984	1.1	3.7

1 Determined using Excel (2003) and individual sample data obtained from Table 5, p.35 of the study report (DER Attachment 2).
2 Determined using first-order kinetics and nonlinear regression analysis (MicroCal Origin v. 3.5; pp. 26-27; Figure 14, p.50).

TRANSFORMATION PRODUCTS: Two major transformation products, 2-chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) and 2-chlorobenzonitrile (M2), were isolated. In addition, M4 was believed to be a solvent artifact of 2-chlorobenzonitrile (M2) since separation of M2 plus M4 by TLC was not possible (pp.29-30; Figures 7-8, pp.43-44). The only minor transformation product identified was 2-chlorobenzamide (M5).

2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) reached a maximum concentration of 42.2% of the applied at 1 day posttreatment and was last detected at 5.1% (single replicate) at 14 days (Table 5, p.35; DER Attachment 2). 2-Chlorobenzonitrile (M2) reached a maximum concentration of 55.6% of the applied at 21 days posttreatment (study termination). The M2/M4 artifact reached a maximum concentration of 40.2% of the applied at 21 days posttreatment. The minor transformation product, 2-chlorobenzamide (M5), reached a maximum of 8.5% of the applied. An unknown minor transformation product (M3) reached a maximum of 4.8% of the applied.

Table 5: Chemical names and CAS numbers for the transformation products of clofentezine.

Applicants Code Name	CAS Number	Chemical Name	Chemical Formula	MW (g/mol)	Smiles String
R1 (M1); AE C593600[1]	--	2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide	--	--	--
R2 (M2)	--	2-Chlorobenzonitrile	--	--	--
R5 (M5)	--	2-Chlorobenzamide	--	--	--

Data obtained from p.16 of the study report.
1 Code name AE C593600 obtained from MRID 47192104.
--: Not reported.

VOLATILIZATION: Volatiles were not collected.

TRANSFORMATION PATHWAY: A transformation pathway was proposed by the study author (p.30; Figure 15, p.51). In this proposed pathway, clofentezine hydrolyzes to 2-chlorobenzoic acid (2-chlorobenzylidene)hydrazide, which is then rapidly hydrolyzed to at least two compounds, identified as 2-chlorobenzonitrile and 2-chlorobenzamide. These two compounds were stable to hydrolysis.

D. PRELIMINARY EXPERIMENT-RESULTS: Overall recoveries were 97.7 ± 2.5% of the applied in the pH 4 buffer, 100.5 ± 1.3% in the pH 7 buffer, and 97.3 ± 4.9% in the pH 9 buffer (Table 2, p.32). Recoveries were low in both the pH 4 and pH 9 buffers at 5 days posttreatment (95.0 and 89.4% of the applied, respectively).

In the **pH 4 buffer**, [^{14}C]clofentezine was relatively stable, decreasing from 99.9% of the applied at time 0 to 95.0% at 5 days posttreatment (Table 3, p.33). No major or minor transformation products were isolated.

In the pH 7 and 9 buffers, [^{14}C]clofentezine hydrolyzed rapidly, forming three transformation products: 2-chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1), 2-chlorobenzonitrile (M2) and 2-chlorobenzamide (M5).

In the **pH 7 buffer**, [^{14}C]clofentezine decreased from 99.5% of the applied at time 0 to 83.3% at 0.1 day posttreatment and 2.8% at 5 days (Table 3, p.33). 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) averaged a maximum of 91.8% of the applied at 1 day posttreatment, decreasing to 81.6% at 5 days. 2-Chlorobenzonitrile (M2) averaged a maximum of 12.3% of the applied at 5 days posttreatment.

In the **pH 9 buffer**, [^{14}C]clofentezine decreased from 93.8% of the applied at time 0 to ≤1.7% at 0.1 and 5 days posttreatment (Table 3, p.33). 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1) averaged a maximum of 93.7% of the applied at 1 day posttreatment, decreasing to 25.1% at 5 days. 2-Chlorobenzonitrile (M2) averaged a maximum of 62.6% of the applied at 5 days posttreatment.

The study author reported that 2-chlorobenzamide (M5) was recovered in the M2 fraction, but did not specify whether this was the case at both pHs and did not provide any quantitative data.

E. SUPPLEMENTARY EXPERIMENT-RESULTS: At 35°C, hydrolysis of [^{14}C]clofentezine occurred more rapidly than hydrolysis at 25°C. Overall recoveries were 101.2 ± 1.7% of the applied (Table 6, p.36; DER Attachment 2). [^{14}C]Clofentezine decreased from 93.5% of the applied at time 0 to 17.8% at 1 day posttreatment and was not detected after 1 day. A half-life was not calculated due to insufficient sampling intervals. The study author estimated graphically a half-life of 0.6 days (pp.27, 29; Figure 1, p.37).

The same transformation products were detected as in the definitive experiment. Four major transformation products were isolated and three were identified. 2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide (M1), a major transformation product, reached a maximum of 83.9% of the applied at 2 days posttreatment and was 9.2% at 14 days (study termination; Table 6, p. 36; DER Attachment 2). 2-Chlorobenzonitrile (M2), a major transformation product, reached a maximum of 57.2% of the applied at 7 days and was 52.2% at 14 days. M4, an HPLC artifact corresponding to 2-chlorobenzonitrile and containing 2-chlorobenzamide, reached a maximum of 42.4% of the applied at 3 days and 36.7% at 14 days (p.30). 2-Chlorobenzamide (M5) reached a maximum of 9.6% at 8 days and was not detected after 8 days. One unknown minor transformation product (M3) was isolated, reaching a maximum of 3.7% of the applied.

Table 6: Hydrolysis of clofentezine, expressed as percentage of the applied (mean ± sd, n = 2), at pH 7 at 35°C.

Compound	**Sampling times (days)**								
	0	1	2	3	4	7	8	10	14
Clofentezine	93.5 ± 4.0	17.8 ± 1.2	--	--	--	--	--	--	--
2-Chlorobenzoic acid (2-chlorobenzylidene) hydrazide (M1)	3.2 ± 1.2	64.1 ± 6.4	83.9 ± 4.6	8.2 ± 0.5	8.3 ± 1.8	5.3 ± 1.5	10.5 ± 0.7	10.4 ± 4.3	9.2 ± 3.0
2-Chlorobenzonitrile (M2)	--	10.7 ± 10.5	7.6 ± 2.0	52.8 ± 7.3	51.7 ± 0.6	57.2 ± 6.5	51.3 ± 6.7	55.1 ± 2.3	52.2 ± 0.5
Unknown (M3)	3.4 ± 2.5	3.7 ± 1.2	--	--	--	--	--	--	3.7, --
M4[1]	--	5.3 ± 0.3	7.1 ± 3.2	42.4 ± 9.4	39.6 ± 3.5	39.6 ± 5.8	30.3 ± 9.6	35.7 ± 4.2	36.7 ± 0.1
2-Chlorobenzamide (M5)	--	--	--, 7.5	--	--	--	9.6 ± 0.2	--	--
CO_2	Volatiles not collected.								
Volatile organics	Volatiles not collected.								
Total Recovery	100.0 ± 0.3	101.5 ± 1.3	102.3 ± 1.9	103.3 ± 1.6	99.6 ± 1.2	102.0 ± 0.8	101.6 ± 2.5	101.1 ± 2.4	99.8 ± 1.0

Means and standard deviations calculated using data obtained from Table 6, p.36 of the study report (DER Attachment 2).

--: Not detected or below limit of quantitation.

1 Corresponds to 2-chlorobenzonitrile due to HPLC artifact and contains 2-chlorobenzamide.

III. STUDY DEFICIENCIES

1. At pH 4, a preliminary study was conducted for 5 days at 49°C. OPPTS and OECD guidelines would not require that a study be conducted at 25°C since <10% of the applied dissipated during the 5 days of incubation.

2. At pH 9, a preliminary study was conducted for 5 days at 49°C. More than 90% of the applied dissipated within 2.4 hours of incubation. A definitive study at 25°C was not performed because clofentezine was considered to be hydrolytically unstable at pH 9 (p.29).

3. The study author reported that M4 likely corresponded to 2-chlorobenzonitrile (M2) and was likely to be an HPLC solvent artifact. M4 could not be separated from M2 in several TLC solvents.

IV. REVIEWER'S COMMENTS

1. The study author indicated that in the 49°C experiment, M2 also contained 2-chlorobenzamide (footnote to Table 3, p.33), but did not elaborate. In the text, the study author only associates M2 with 2-chlorobenzoic acid (p.29).

2. Test solutions (2.10 μg a.i./L) were prepared at approximately the water solubility (2.5 μg a.i./L) of clofentezine (p.18).

3. The unlabeled reference standard, 2-chlorobenzonitrile (M2) could not be visualized by quenching of UV light or in an iodine chamber (p.23).

V. REFERENCES

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 161-1. Hydrolysis studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

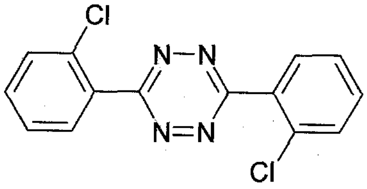
3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.

Attachment 1: Structures of the Parent Compound and Transformation Products

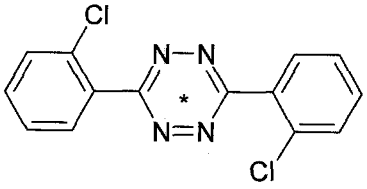
Clofentezine [NC 21314, NC 21 314, AE B084866]

IUPAC Name:	3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS Name:	3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS Number:	74115-24-5.
SMILES String:	Clc1ccccc1c2nnc(c3ccccc3Cl)nn2 (EPI Suite, v3.12 SMILES).

Unlabeled



[1,2,4,5-Tetrazine-^{14}C]Clofentezine

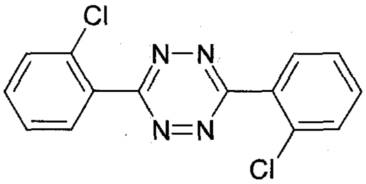


* = Location of the radiolabel.

Identified Compounds

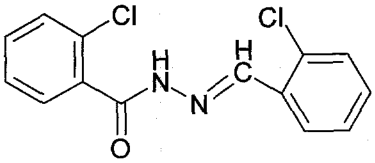
Clofentezine [NC 21314, NC 21 314, AE B084866]

IUPAC Name: 3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS Name: 3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine.
CAS Number: 74115-24-5.
SMILES String: Clc1ccccc1c2nnc(c3ccccc3Cl)nn2 (EPI Suite, v3.12 SMILES).



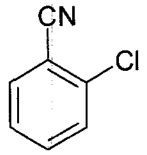
AE C593600 [R1, M1, Compound III]

IUPAC Name: 2-Chlorobenzoic (2-chlorobenzylidene) hydrazide.
2-Chlorobenzoic acid (2-chlorobenzylidene)hydrazide.
CAS Name: 2-Chlorobenzoic acid {(2-chlorophenyl)methylene}hydrazide.
CAS Number: Not reported.



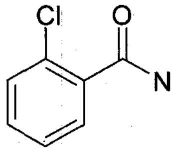
AE F023666 [R2, M2]

IUPAC Name: 2-Chlorobenzonitrile.
CAS Name: Not reported.
CAS Number: Not reported.



AE F092117 [R5, M5]

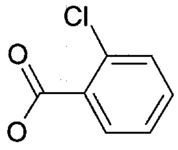
IUPAC Name: 2-Chlorobenzamide.
CAS Name: Not reported.
CAS Number: Not reported.



Unidentified Reference Compounds

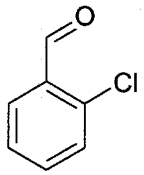
NC 233 [R3, Compound V]

IUPAC Name: 2-Chlorobenzoic acid.
CAS Name: Not reported.
CAS Number: Not reported.



2-Chlorobenzaldehyde [R4]

IUPAC Name: 2-Chlorobenzaldehyde.
CAS Name: Not reported.
CAS Number: Not reported.



Attachment 2: Excel Spreadsheets

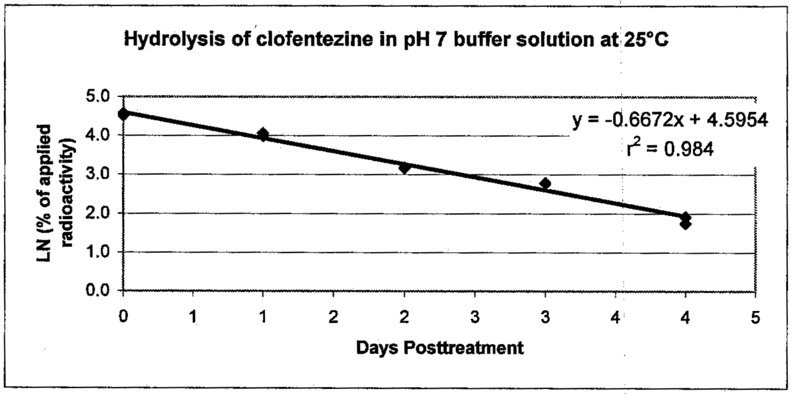
Chemical: Clofentezine
MRID: 47192107
PC Code: 125501
Guideline: 161-1

Recovery of Radioactivity after Incubation at 25°C
pH 7

Days posttreatment	Clofentezine	
	% of applied	ln (% of applied)
0	90.6	4.5065
0	96.3	4.5675
1	57.3	4.0483
1	53.4	3.9778
2	23.7	3.1655
2	23.5	3.1570
3	16.1	2.7788
3	15.9	2.7663
4	5.7	1.7405
4	6.7	1.9021

Data imported from distribution worksheet.

Half-life (days):
1.0



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.991967768
R Square	0.984000053
Adjusted R Square	0.98200006
Standard Error	0.134516956
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	8.90267732	8.902677	492.0016598	1.80356E-08
Residual	8	0.144758492	0.018095		
Total	9	9.047435813			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.59538921	0.073677971	62.37128	4.85433E-12	4.425487394	4.76529103	4.425487394	4.765291027
X Variable 1	-0.667183532	0.030078906	-22.1811	1.80356E-08	-0.736545659	-0.59782141	-0.736545659	-0.597821406

Chemical: Clofentezine
MRID: 47192107
PC Code: 125501
Guideline: 161-1
pH 7

Recovery of Radioactivity after Incubation at 25°C
Data obtained from Table 5, p. 35 of the study report.

Total

Days posttreatment	% applied radioactivity		
	Total	Average	SD
0	99.8	100.0	0.3
0	100.2		
1	104.3	103.0	1.8
1	101.7		
2	101.7	101.3	0.6
2	100.9		
3	101.6	100.8	1.1
3	100.0		
4	101.5	101.5	0.0
4	101.5		
7	101.3	100.6	1.0
7	99.9		
10	101.3	102.0	0.9
10	102.6		
14	102.6	102.0	0.8
14	101.4		
21	103.0	102.9	0.2
21	102.7		
Total		101.6	1.2

Clofentezine

Days posttreatment	% applied radioactivity		
	Clofentezine	Average	SD
0	90.6	93.5	4.0
0	96.3		
1	57.3	55.4	2.8
1	53.4		
2	23.7	23.6	0.1
2	23.5		
3	16.1	16.0	0.1
3	15.9		
4	5.7	6.2	0.7
4	6.7		
7		#DIV/0!	#DIV/0!
7			
10		#DIV/0!	#DIV/0!
10			
14		#DIV/0!	#DIV/0!
14			
21		#DIV/0!	#DIV/0!
21			

M1

Days posttreatment	% applied radioactivity		
	M1	Average	SD
0	4.0	3.2	1.2
0	2.3		
1	41.7	42.2	0.6
1	42.6		
2	39.2	39.0	0.3
2	38.8		
3	9.3	9.3	0.1
3	9.2		
4	13.4	9.6	5.4
4	5.7		
7	5.1	5.6	0.6
7	6.0		
10	6.8	8.2	2.0
10	9.6		
14		5.1	#DIV/0!
14	5.1		
21		#DIV/0!	#DIV/0!
21			

Chemical: Clofentezine
MRID: 47192107
PC Code: 125501
Guideline: 161-1
pH 7

Recovery of Radioactivity after Incubation at 25°C
Data obtained from Table 5, p. 35 of the study report.

M2

Days posttreatment	% applied radioactivity		
	M2	Average	SD
0		#DIV/0!	#DIV/0!
0			
1	3.3	2.3	1.4
1	1.3		
2	17.0	17.0	0.1
2	16.9		
3	39.3	39.0	0.4
3	38.7		
4	46.3	47.6	1.8
4	48.9		
7	47.6	49.7	3.0
7	51.8		
10	53.4	53.9	0.6
10	54.3		
14	51.1	48.9	3.1
14	46.7		
21	55.6	55.6	0.1
21	55.5		

M3

Days posttreatment	% applied radioactivity		
	M3	Average	SD
0	5.2	3.4	2.5
0	1.6		
1	2.0	1.6	0.6
1	1.2		
2		#DIV/0!	#DIV/0!
2			
3	4.7	4.7	0.1
3	4.6		
4	2.5	2.2	0.5
4	1.8		
7		#DIV/0!	#DIV/0!
7			
10	3.2	3.2	#DIV/0!
10			
14	5.7	4.8	1.3
14	3.9		
21		#DIV/0!	#DIV/0!
21			

M4

Days posttreatment	% applied radioactivity		
	M4	Average	SD
0		#DIV/0!	#DIV/0!
0			
1		3.2	#DIV/0!
1	3.2		
2	18.3	20.0	2.4
2	21.7		
3	26.0	25.8	0.3
3	25.6		
4	26.8	29.2	3.4
4	31.6		
7	42.0	38.8	4.6
7	35.5		
10	31.8	31.3	0.7
10	30.8		
14	37.1	37.4	0.4
14	37.6		
21	40.7	40.2	0.8
21	39.6		

M5

Days posttreatment	% applied radioactivity		
	M5	Average	SD
0		#DIV/0!	#DIV/0!
0			
1		#DIV/0!	#DIV/0!
1			
2	3.5	3.5	0.0
2	3.5		
3	6.1	6.1	0.1
3	6.0		
4	6.8	6.8	0.1
4	6.7		
7	6.6	6.6	0.0
7	6.6		
10	6.1	7.0	1.2
10	7.8		
14	8.7	8.5	0.3
14	8.3		
21	6.7	7.2	0.6
21	7.6		

Chemical: Clofentezine
MRID: 47192107
PC Code: 125501
Guideline: 161-1
pH 7

Recovery of Radioactivity after Incubation at 35°C
Data obtained from Table 6, p. 36 of the study report.

Total

Days posttreatment	% applied radioactivity		
	Total	Average	SD
0	99.8	100.0	0.3
0	100.2		
1	100.6	101.5	1.3
1	102.4		
2	100.9	102.3	1.9
2	103.6		
3	102.1	103.3	1.6
3	104.4		
4	98.7	99.6	1.2
4	100.4		
7	101.4	102.0	0.8
7	102.5		
8	99.8	101.6	2.5
8	103.4		
10	99.4	101.1	2.4
10	102.8		
14	99.1	99.8	1.0
14	100.5		
Total		101.2	1.7

Clofentezine

Days posttreatment	% applied radioactivity		
	Clofentezine	Average	SD
0	90.6	93.5	4.0
0	96.3		
1	18.6	17.8	1.2
1	16.9		
2		#DIV/0!	#DIV/0!
2			
3		#DIV/0!	#DIV/0!
3			
4		#DIV/0!	#DIV/0!
4			
7		#DIV/0!	#DIV/0!
7			
8		#DIV/0!	#DIV/0!
8			
10		#DIV/0!	#DIV/0!
10			
14		#DIV/0!	#DIV/0!
14			

M1

Days posttreatment	% applied radioactivity		
	M1	Average	SD
0	4.0	3.2	1.2
0	2.3		
1	68.6	64.1	6.4
1	59.5		
2	87.1	83.9	4.6
2	80.6		
3	8.5	8.2	0.5
3	7.8		
4	9.5	8.3	1.8
4	7.0		
7	4.2	5.3	1.5
7	6.3		
8	11.0	10.5	0.7
8	10.0		
10	7.3	10.4	4.3
10	13.4		
14	7.0	9.2	3.0
14	11.3		

Chemical: Clofentezine
MRID: 47192107
PC Code: 125501
Guideline: 161-1
pH 7

Recovery of Radioactivity after Incubation at 35°C
Data obtained from Table 6, p. 36 of the study report.

M2

Days posttreatment	% applied radioactivity		
	M2	Average	SD
0		#DIV/0!	#DIV/0!
0			
1	3.3	10.7	10.5
1	18.1		
2	9.0	7.6	2.0
2	6.2		
3	57.9	52.8	7.3
3	47.6		
4	52.1	51.7	0.6
4	51.3		
7	61.8	57.2	6.5
7	52.6		
8	56.0	51.3	6.7
8	46.5		
10	53.5	55.1	2.3
10	56.7		
14	51.8	52.2	0.5
14	52.5		

M3

Days posttreatment	% applied radioactivity		
	M3	Average	SD
0	5.2	3.4	2.5
0	1.6		
1	4.5	3.7	1.2
1	2.8		
2		#DIV/0!	#DIV/0!
2			
3		#DIV/0!	#DIV/0!
3			
4		#DIV/0!	#DIV/0!
4			
7		#DIV/0!	#DIV/0!
7			
8		#DIV/0!	#DIV/0!
8			
10		#DIV/0!	#DIV/0!
10			
14	3.7	3.7	#DIV/0!
14			

M4

Days posttreatment	% applied radioactivity		
	M4	Average	SD
0		#DIV/0!	#DIV/0!
0			
1	5.5	5.3	0.3
1	5.1		
2	4.8	7.1	3.2
2	9.3		
3	35.7	42.4	9.4
3	49.0		
4	37.1	39.6	3.5
4	42.1		
7	35.5	39.6	5.8
7	43.7		
8	23.5	30.3	9.6
8	37.1		
10	38.6	35.7	4.2
10	32.7		
14	36.6	36.7	0.1
14	36.7		

M5

Days posttreatment	% applied radioactivity		
	M5	Average	SD
0		#DIV/0!	#DIV/0!
0			
1		#DIV/0!	#DIV/0!
1			
2		7.5	#DIV/0!
2	7.5		
3		#DIV/0!	#DIV/0!
3			
4		#DIV/0!	#DIV/0!
4			
7		#DIV/0!	#DIV/0!
7			
8	9.4	9.6	0.2
8	9.7		
10		#DIV/0!	#DIV/0!
10			
14		#DIV/0!	#DIV/0!
14			

Attachment 3: Transformation Pathway Presented by Registrant

Figure 15: Metabolic pathway of the hydrolysis of Clofentezine at pH 7