Environmental Chemistry Review for N-N' [1,4-piperazinediyl bis (2,2,2-tricloroethylidene)] bis [Formamide]
(Triforine)
Reg. No. 279-EOON

Reviewed 11/10/75

F.M.C. Corp
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I. INTRODUCTION

1. CA-70203, Cela W 524 EC, FMC-28221

2. Roses (Fungicide) Greenhouse

3. See new chemical reviews dated 3-23-72 and 11-7-72.

4. Originally submitted by Cela Merck gmbh., EPA temporary permit 6967-EXP-1G and later by FMC under 279-EXP-50G.

5. Full registration is proposed for non crop use.

II. DIRECTIONS FOR USE

Roses-Greenhouse-Apply 10-12 oz (7.94-9.53 oz ai) per 100 gal. water Repeat every 7-10 days as necessary.

III. DISCUSSION OF DATA

1. Soil Degradation of Triforine
(Impact of Triforine on the environment, addendum 1, Ref. #1)

Aerobic Metabolism

^{14}C and ^{3}H labelled triforine were added to both sandy loam and silt loam soils. At 2 and 20 ppm water was added to soil to 70% moisture level, and soils were incubated in biometer flasks at 25-30° C.

Prior to treatment the microbial levels of the soils were evaluated with a respirometer, and they were found to be "microbiologically active."

Labels

Tritium label was on H attached to ring carbons. Carbon labels were on both carbons of the two tri-chloro ethylene groups.

Anaerobic Metabolism

Samples of Silt loam and sandy loam soil treated at 2 ppm with ^{3}H-triforine were maintained in flasks for 30 days under aerobic, followed by 30 and 60 day incubations under nitrogen.

Sterile Soil Metabolism

Sandy loam and silt loam soils were sterilized by dry heat, water was added aseptically to 70% moisture. Soil was treated at 2 ppm with ^{3}H-triforine and incubated 30 days.

Analytical Procedure

Soil was extracted 2X with 90% methanol/water. The extract was partitioned with methylene chloride. The organic and polar phases of the partition were subjected to TLC autoradiography. Solid soil residue was analyzed by combustion.

The nature of bound residues were analyzed by fractionation of the humin, humic acid and fulvic acid fractions.

Radioactive triforene was partitioned between hexane and acetonitrile for the determination of p-value.

Soil Characteristics

	%Sand	%Silt	%Clay	%O.M.	Moisture	C.E.C.	pH
Sandy Loam	75.2	18.0	6.8	3.1	13.0	7.3	6.1
Silt Loam	25.2	51.6	23.2	3.9	22.2	16.8	4.9

Soil metabolism data was presented in a series of tables which have been combined below.

The Fate of Radiolabelled Triforine in Soil

Condition	Aerobic	Aerobic	Aerobic	Anerobic	Sterile	Sterile
Label	^{3}H	^{3}H	^{14}C	^{3}H	^{3}H	^{3}H
Soil Type	Sandy L	Silt L	Sandy L	Silt L	Sandy L	Silt L
Days	60	60	90	90	30	30
ppm Applied compound	20	20	20	20	2	2
	% of Radioactivity Present					
Triforine	31.9	11.1	16.9	5.1	29.6	29.5
FMC-29746	0.3	0				
FMC-29745	0.7	0.3	0.9		2.9	2.3
FMC-29748	7.3	4.0	0.5			
FMC-29747			1.3			
Unidentified	16.2	15.0		0.55	1.3	2.1
Non-Extractable	43.7	69.5	34.3	71.0	58.3	54.8
CO_2 evolved			37.9			
Polar Compounds				23.4	7.9	11.3

Distribution of ^{3}H residues in aerobic soil (2 ppm)

% Distribution

Time (Days)	Sandy Loam Organic	Polar	Bound	Silt Loam Organic	Polar	Bound
0	90.4	4.6	5.0	87.1	1.7	11.2
7	72.6	5.8	21.7	35.1	11.7	33.8
14	59.3	9.6	31.1	43.9	10.8	45.2
30	44.1	19.4	36.5	35.7	22.1	42.3
60	44.5	13.0	42.6	18.0	17.1	65.0

Results

1. 66 and 89% of bound ^{3}H was released by HCl and NaOH respectively in fractionation procedure.

2. About 75% of the bound ^{14}C and ^{3}H was in the fulvic acid, 1.6-15.3% was in the humin and 4.5-16.8% was in the humic acid.

3. Triforine was degraded in sterile soil at 30 days to FMC-29745 and some unidentified polar and organic compounds.

4. P value - Hexane-acetonitrile = 0.001
   P value - Benzene-water = 0.50

Comparison of Aerobic, Anerobic and Sterile Studies

	Aerobic	Anerobic	Sterile
Extractable degradation products	Yes	No	Yes
Polar Compounds	No	Yes	Yes
Bound 30 to 40%	Yes	Yes	Yes
Bound over 50%	No	Yes	Yes
Unidentified extractable compounds	Yes	No	Yes

Conclusions

1. Percentages on soil metabolism tables were percent found at that particular time. They should have been percent of radioactivity applied. We must have this information.

2. Bound residues were high and increased with time.

3. Supporting data for radiolabel studies (counting time, possible error, counting efficiency, dpm. etc.) was not included.

4. Sterile soil results show that triforine can be degraded to polar compounds under aseptic conditions.

2. Leaching of ^{3}H-Triforine Residues in Cosad Sandy Loam Soil (Impact of Triforine on the Environment, addendum; Ref. #2)

A 0.3 x 12" column was packed with sandy loam and saturated. Study was divided into leaching of organo-soluble and bound residues studies. Ten microliters of methylene chloride extract from a 20 ppm 30 day aerobic metabolism study (see previous study) was applied to the column. Extract counted at 334,000 dpm. For the bound residue study, 1 gram (1 x 10^6 dpm) of [redacted] Cosad sandy loam bound residues were applied to the top of the columns.

Each column was treated with 0.5" water per day. After 45 days, each column was cut into 24 one half inch segments, and were analyzed by combustion.

Distribution of ^{3}H Triforine Residues

Segment	% Distribution in Column Organo-Soluble	Bound
0-3"	27.2	17.0
3-6"	10.3	4.9
6-9"	8.3	2.6
9-12"	5.1	1.6
Effluent	49.1	47.9
Bound Residue Soil Segment	----	26.0

Soil characteristics: % sand 75.2, % salt 18.0, % day 6.8, % O.M. 3.1, moisture 13.0, CEC 7.3, pH 6.1

Conclusions

1. Applicant did not state what amount of Triforine was applied to columns. We must have this.

2. Since no data was given linking dpm to ppm, it was not possible to determine the amount applied.

3. Both applications resulted in 50% of the applied radioactivity being recovered from the leachate water.

4. Environmental uses of Triforine may present serious leaching problems.

5. We have no definite proof of whether soil applied in bound residue study was first extracted with organic solvents.

6. We need to know why a methylene chloride extract was applied to the soil when methylene chloride was not shown to be a good extracting solvent and other soil studies used methanol/water.

3. Comparative Radio-Thin Layer Chromatographic Investigations of the Decomposition of $^{3}H/^{14}C$-W-524 (Triforene) in Aqueous Solution (Reference #3)

30 ppm of ^{14}C (labelled on both carbons of both trichloro-ethylene moieties) and ^{3}H (uniform ring label) were incubated at room temperature in non-buffered aqueous solution in sealed flasks. The pH of aqueous solution was initially 6.2 and rapidly dropped to 3.2. Flasks were exposed to diffused daylight.

Solutions were counted by LSC. $^{14}CO_2$ was trapped with NaOH.

Aliquots were freeze dried, dissolved in acetone/water, and analyzed by TLC with co-chromatography of parent, and UV and radiometric detection. Spots were scraped and counted.

% of Applied Radioactivity Recovered As Parent Compound

Time (days)	^{3}H	^{14}C
0	100	100
2	49	53
4	35	28
6	20	20
10	10	11
14	6	11
22	2	4

Results

1. Registrant stated that only minimal quantities of radioactivity were trapped in the NaOH.

2. On TLC run showed 9 degradation products and no parent compound after 22 days.

3. The main degradation product (unidentified) comprised 40-80% of the radioactivity present. It contained both ^{3}H and ^{14}C labels.

4. After an initial increase in ^{3}H, the amounts of ^{3}H and ^{14}C at the origin increased at the same rate and remained at a similar level.

Conclusions

1. Study was not carried out at pH 5, 7 and 9.

2. Some photodegradation may have taken place since solution was not shielded from light.

3. Registrant stated some degradation took place before the start and starting material was 95% pure.

4. Registrant concluded that drop in pH was the result of HCl being formed by hydrolysis.

5. Results indicate that degradation products probably contain both ^{14}C and ^{3}H labels.

4. Decomposition Products of Radioactive Triforine in Aqueous Solution.

30 ppm non-buffered aqueous solutions of ^{14}C (labelled on both carbons of trichloroethylene moiety) and ^{3}H (uniform ring label) Triforene were incubated at room temperature, in sealed flasks, exposed to diffused daylight for period of 5-6 days and 13 weeks.

Aliquots were freeze dried and the residue was subjected to TLC with co chromatography and identification of spots by a methane flow through counter. Spots were scraped and analyzed by mass spec.

Results

1. Compounds isolated after 6 days were:

   FMC-29745, FMC-29748, Triforine and FMC-29747

2. The main degradation product was not identified. It was very unstable and was changed into FMC-29745 possibly by the experimental conditions.

3. After 13 weeks no parent compound was detectable.

4. After separation and processing of the 13 week sample, a 50% loss of total radioactivity was noted. Since no radioactivity was detected in the lab, glassware or equipment, registrant concludes the loss could be due to a volatile product.

5. Main degradation products after 13 weeks were mono and bis glyoxal piperqzine, also other piperazine compounds (Structures, but no names or other designations provided) and 3-trichloro, 2-Keto, propionaldehyde were formed.

Conclusions

1. After 13 weeks hydrolysis at pH 3.2, the Triforine had been completely degraded, mainly to mono or bis glyoxal piperazine.

2. Registrant concluded that the compound W-1084, may have been the result of photodegradation.

3. The 50% loss of total radioactivity could not be accounted for by volatilization, since the experiment was carried out in a sealed flask.

IV. CONCLUSIONS

1. The hydrolysis study was not carried out at pH 5, 7 and 9 and was not adequately shielded from light.

2. Explanation is needed to determine if the percentages reported in the soil metabolism study were for % residue at the time of analysis or percent residue relative to the amount applied.

3. Supporting data for the radiolabel studies (counting time, possible error, counting efficiency etc.) was not included in any of the studies.

4. It was not possible to determine the amount or rate of application of Triforine in the leaching study.

5. Triforine binds strongly to sandy loam and silt loam soils. Under aerobic, anaerobic and sterile conditions, 55-71% of applied ^{3}H was bound.

6. Triforine is a strong leacher, with 50% of applied ^{3}H being recovered in the leachate water.

V. RECOMMENDATIONS

A. Object to Registration

1. Further clarification of the soil metabolism studies (Reference #1, Addendum I, Impact of Triforine on the Environment; Sep. 1974) is needed. Tables 3, 4, 7, 8, 9, 10 and appendices 1, 2 and 3 must be amended to include the actual amount in ppm actually found in each fraction of each sample at each sampling interval in both soil types under aerobic, anaerobic and sterile conditions. The data should be expounded on the basis of percentage of amount initially applied rather than on a relative percentage distribution within a subsample. Some of the bargraphs are misleading in that they imply total accountability of initially applied amount whereas they actually demonstrate relative accountability of recoverable labels at each sampling interval. The laboratory data, including cpm, dpm, background, may also be helpful in the evaluation of this study. This study may not be acceptable for outdoor uses but, this can only be determined after our questions have been answered.

2. Several questions need to be answered on the leaching study.

   a. Methylene chloride has not been shown to be a good extracting solvent. Other soil studies use methanol/water. How do we know that all the extractable residues have been removed from the soil for the leaching study of degradation products?

b. In the bound leaching study was the soil extracted first? It appears not, as the results are similar to the organo-soluble leaching study.

c. This study will not be acceptable for any outdoor applications. See enclosure for acceptable leaching study.

3. A hydrolysis study is needed. See enclosure.

4. Other uses of this chemical will require additional data on fate of this chemical in the environment. This information would include, but not strictly be limited to, a fish accumulation study, effects on or by microbes, plant uptake by subsequent crops if use involves crop areas, field persistence study and field leaching and runoff study.

REN 2/21/75

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