

Date Out:

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ENVIRONMENTAL FATE AND GROUND WATER BRANCH

Review Action

OCT 4 1993

To: Dennis Edwards, PM #19
Registration Division (H7507C)

From: Akiva D. Abramovitch, Ph.D., Head
Chemistry Review Section 3
Environmental Fate and Effects Ground Water Branch/EFED (H7507C)

Thru: Henry Jacoby, Chief
Environmental Fate & Ground Water Branch/EFED (H7507C)

Attached, please find the EFGWB review of AC 303,630 (also known as CL 303,630).

Common Name:	None	Trade name:	PIRATE
Company Name:	American Cyanamide Company		
ID #:	129093		
Purpose:	Review of studies for an EUP.		

Type Product:	Action Code:	EFGWB #(s):	Review Time:
Insecticide-Miticide	710 EUP-NC-F/F USE	93-0823	

STATUS OF STUDIES IN THIS PACKAGE:

Guideline #	MRID	Status ¹
161-1	42770240	A
161-2	42770241	*
161-3	42770242	A
162-1	42770243	A
163-1	42770244	U
165-4	42770245	A

STATUS OF DATA REQUIREMENTS ADDRESSED IN THIS PACKAGE:

Guideline #	Status ²
161-1	S
161-2	*
161-3	S
162-1	S
163-1	P
165-4	S

¹Interim Report, Not Reviewed Pending Completion of Study

²Study Status Codes: A=Acceptable U=Upgradeable C=Ancillary I=Invalid

Data Requirement Status Codes: S=Satisfied P=Partially satisfied N=Not satisfied R=Reserved W=Waived

1. CHEMICAL

Common Name: None Given

Chemical Identification and Designations:

American Cyanamide Company: AC 303,630 and CL 303,630

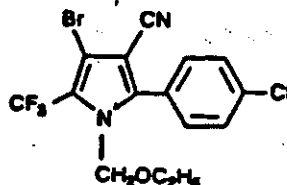
CAS Registry No.: 122453-73-0

CA Name: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile

IUPAC Name: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)pyrrole-3-carbonitrile

Trade name(s): PIRATE

Structure:



Formulations: Suspension Concentrate (SC)

Physical/Chemical properties:

Molecular formula:	C ₁₅ H ₁₁ BrClF ₃ N ₂ O
Molecular weight:	407.6
Physical state:	white to off-white powder
Melting point:	100-101°C
Vapor pressure (25°C):	<1.3 x 10 ⁻⁵ Pa (<1.0 x 10 ⁻⁷ torr)
Solubility:	0.12 mg/L water (25°C); sol. in methanol, acetonitrile, acetone, ethyl acetate

2. TEST MATERIAL

All studies: Active Ingredient

3. STUDY/ACTION TYPE

New chemical EUP: data evaluation and review of 1) hydrolysis, 2) aqueous photolysis, 3) soil photolysis, 4) aerobic soil metabolism, 5) Ads/Des (batch) on soils, 6) Accumulation in Fish.

4. STUDY IDENTIFICATION

STUDY MRID 42770240

Mangels, G. 1993. AC 303,630: Hydrolysis.
Laboratory Project ID: ENV 93-006. Unpublished study performed and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ.

STUDY MRID 42770241

Mangels, G. 1993. CL 303,630: Photodegradation in Water--Interim Report. Laboratory Project ID: ENV 93-017. Unpublished study performed and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ.

STUDY MRID 42770242

Mangels, G. 1993. AC 303,630: Photodegradation on Soil. Laboratory Project ID: ENV 93-014. Unpublished study performed and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ.

STUDY MRID 42770243

Mangels, G. 1993. AC 303,630: Aerobic Soil Metabolism. Laboratory Project ID: ENV 93-013. Unpublished study performed and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ.

STUDY MRID 42770244

Mangels, G. 1993. AC 303,630: Adsorption/Desorption on Soils. Laboratory Project ID: PD-M Volume 29-14. Unpublished study performed and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ.

STUDY MRID 42770245

Zulalian, Jack. 1993. CL 303,630: Uptake, Depuration, Bioconcentration and Metabolism of Carbon-14 CL 303,630 in Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Test Conditions. Laboratory Project ID: American Cyanamid No. MET 93-002, ABC Laboratories No. 39603-2. Unpublished study performed by American Cyanamid Company, Ag. Res. Div., Box 400, Princeton, NJ, and ABC Laboratories Inc., Aquatic Toxicology Programs Div., 7200 East ABC Lane, Columbia, MO. Submitted by American Cyanamid Company, Agricultural Research Division, Box 400, Princeton, NJ.

5. DATA EVALUATED AND REVIEWED BY:

Alex Clem
Environmental Scientist, Rev. Sec. 3
EFGWB/EFED/OPP

Signature: Alex Clem
Date: OCT 1 1993

6. APPROVED BY:

Akiva D. Abramovitch
Head, Review Section 3
EFGWB/EFED/OPP

Signature: Akiva Abramovitch
Date: OCT 1 1993

7. CONCLUSIONS

161-1. Hydrolysis. MRID 42770240: Satisfied.

AC 303,630 proved stable against hydrolysis at pH 5, 7 and 9 (half-life indeterminately long in a 30 day test period). There was no evidence of trace hydrolysates.

161-2. Photodegradation in Water. MRID 42770241: Interim Report.

This interim report was not evaluated or reviewed pending its completion, and is not required for an EUP. The registrant concluded that AC 303,630 has a half-life of about one week for this process, with several photoproducts to be identified.

161-3. Photodegradation on Soil. MRID 42770242: Satisfied.

AC 303,630 was found to photolyze with a $T(1/2)$ of 75 ± 21 days for continuous irradiation with a filtered xenon-arc source. Two identified photoproducts, the desethoxymethyl compound (CL 303,268) and 2-(4-chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-2-pyrrolidine-3-carbonitrile (CL 325,195), each accounted for about 5% (0.8 ppm) of the original dose after 30 days. Six possible candidates for unknown substances, none contributing more than about 3% (0.5 ppm), were eliminated. About 85% of the total dose present after 30 days was identified; several unidentified components probably exceeded 0.01 ppm. Depending on results of other studies, further work on the chemistry and kinetics of products could be necessary before a definitive environmental fate assessment is made.

162-1. Aerobic Soil Metabolism. MRID 42770243: Satisfied.

At the end of a one year test period, approximately 82% of parent AC 303,630 remained, corresponding to a $T(1/2)$ of 3.8 years. The only metabolite ever found to be present at concentrations >0.01 ppm was identified as the desbromo compound, and appeared to have accumulated gradually to approximately 8% (0.08ppm) of the total dose by the end of the study. Several unidentified substances, each of which accounted for less than 1% (0.01ppm) of the parent, were detected. Trapped $^{14}\text{CO}_2$ was

insignificant. No further work on metabolite chemistry or kinetics was stated.

163-1. ADS/DES on Soils. MRID 42770244: Upgradeable.

In spite of shortcomings in the reporting of experimental procedures and calculation of results (see recommendations below, and conclusion and comment sections of DER), it was clear from this batch equilibrium study that AC 303,630 bound to the four test soils for each of the four test concentrations. ADS/DES parameters need to be recalculated, but values for $K_d(ads)$, $K_d(des)$ and K_f should be in the ranges of 30-200, 80-400 and 20-200, respectively, depending on soil class; K_{oc} was similar for all soils and should average around 14000.

Coupled with its low aqueous solubility (0.12-0.14 ppm) over a wide pH range (pH 4-10) this compound is expected to be immobile in soils.

165-4. Accumulation in Fish. MRID 42770245: Satisfied.

In spite of shortcomings in the study and in the reporting of experimental results (see recommendations below, and conclusion and comment sections of DER), essential conclusions have been gained.

During 33 days of uptake at a nominal concentration of 1 ppb (1/10 of LC_{50}), the highest observed total bioconcentration factors for AC 303,630 and its residues in bluegill sunfish were 3900X in viscera, 2300X in whole fish and 1200X in fillet. After a 21 day depuration, a tight average of approximately 97% of residues in all tested parts of the fish had been eliminated, corresponding to a depuration half-life of roughly 4 days. One major and two minor metabolites were identified and quantified in aquaria waters. The same major identified metabolite, the desbromo derivative, was present in both the aquaria waters and fish tissues, and accounted for 89-95% (600-3400 ppb) of the residues in fish tissues; parent compound accounted for only 2-3% (10-100 ppb). Minor polar, but unidentified metabolites accounted for 2-4% (10-140 ppb) of these tissue residues.

Environmental Fate Assessment

AC 303,630 is a persistent chemical (aerobic soil metabolism half-life 3.8 years), has low solubility in water (0.12-0.14 ppm over the pH range 4-10) and binds strongly to soils (K_{oc} roughly 14000 for all soils tested). It is stable against hydrolysis; has a soil photolysis half-life of about 75 days with two identified degradates each present at about 5% at the end of 30 days, and several lesser, unidentified degradates (see conclusions above); has one aerobic soil metabolite present at about 8% after one year with several lesser, unidentified degradates (see conclusions above). An unevaluated interim report concluded that AC 303,630 has an aqueous photolysis half-life of about one week with several photoproducts to be identified.

At least three of the above degradates are distinct. Evidence from the experiments suggests slow, long-term accumulation of these parent-like

product molecules, indicating that these unfragmented degradates may also be persistent. There is collateral evidence from extraction procedures that at least one degradate also binds to soil. No direct data has been presented on degradate chemistry or mobility.

AC 303,630 is moderately toxic to bluegill sunfish, accumulating (up to 3900X in viscera, 2300X in whole fish and 1200X in fillet) as the desbromo metabolite (up to 95%). In the unlikely event it reaches fish, it is depurated with a half-life of roughly 4 days.

For the intended use pattern (see background section below) aerobic soil metabolism may be the only significant mode of breakdown. Soil photolysis and aqueous photolysis would probably not be important modes of environmental dissipation. Thus adsorption to soil is the principal near to long-term environmental fate of AC 303,630. For a more definitive environmental fate assessment, data is needed for the mobility of the major aerobic soil degradate, the desbromo compound CL 312,094.

8. RECOMMENDATIONS

EFGWB has sufficient data for the EUP. However, before further registration actions are submitted to this branch the registrant should do the following:

1) To avoid confusion, reference the subject chemical in the same way in all studies. In various studies either AC 303,630 or CL 303,630 or both have been used without distinction.

2) Perform an appropriate ADS/DES study for the aerobic soil metabolite, CL 312,094.

3) Provide a complete and corrected record of the ADS/DES study (MRID 42770244), as indicated in the comments section of the DER, by submitting the appropriate recalculated results, tables and figures, and supplying their own material balance.

4) Note the detailed comments at the end of the DER for the disorganized, merged accumulation in fish study (MRID 42770245). As appropriate, heed, respond or comply. Primarily reconcile items 2 and 3 there, and accordingly provide changes to the report. No further lab work for this study should be necessary.

5) In the future, base concentration computations on actual weights used in experiments, not some hypothetical field use rate (for example, see comment 2 in the DER for study 161-3, MRID 42770242).

9. BACKGROUND

A. Introduction

CL 303,630 (PIRATE™) is a new chemical stated to be an insecticide-miticide with special benefits for cotton, but which may

also have uses in vegetable and fruit crops. It is based on the pyrrole nucleus, and is touted to represent a new class of pesticide chemicals with a unique mode of biological action--uncoupling oxidative phosphorylation (disruption of the energy transforming mechanism). PIRATE is also claimed to show no indication of cross-resistance in insects or mites which are resistant to a host of other classes of compounds.

The objective of the EUP is to evaluate PIRATE in cotton on a broad spectrum of 21 target pests (especially the tobacco budworm) on a total of about 4000 acres in 11 cotton growing states, and to investigate its role in IPM. It has been studied in small plot trials since 1989. The EUP is planned for the 1994 and 1995 growing seasons.

B. Directions for Use

PIRATE is to be applied as a foliar spray (suspension concentrate) at rates of about 0.1 to 0.4 lb/acre, depending on the pest, with a minimum of 5 gallons of water per acre with ground equipment or 2 gallons of water per acre by aircraft.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES

Refer to attached reviews.

11. COMPLETION OF ONE-LINER

A "one-liner" will be initiated.

12. CBI APPENDIX

All data reviewed here are considered "company confidential" by the registrant and must be treated as such.

AC 303,630

(Also Known as CL 303,630 and PIRATE™)

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6. Accumulation in Fish.	6.1
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Pages 10 through 12 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
 - ☐ Identity of product impurities.
 - ☐ Description of the product manufacturing process.
 - ☐ Description of quality control procedures.
 - ☐ Identity of the source of product ingredients.
 - ☐ Sales or other commercial/financial information.
 - ☐ A draft product label.
 - ☐ The product confidential statement of formula.
 - ☐ Information about a pending registration action.
 - ☒ FIFRA registration data.
 - ☐ The document is a duplicate of page(s) _____.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

STUDY 1

CHEM 129093

AC 303,630

HYDROLYSIS (\$161-1)

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID (MRID) 42770240

Mangels, G. 1993. AC 303,630: Hydrolysis.

Laboratory Project ID: ENV 93-006. Unpublished study performed and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ.

REVIEWED BY: Alex Clem

TITLE: Environmental Scientist

ORG: EFGWB/EFED/OPP/OPPTS/EPA

TEL: 703-305-5991

SIGNATURE: Alex Clem

CONCLUSIONS

1) This study satisfies EPA data requirements for hydrolysis.

2) AC 303,630 (also designated CL 303,630), a compound of low aqueous solubility (0.13ppm), proved stable against hydrolysis at pH 5, 7 and 9 (half-life indeterminately long in a 30 day test period).

MATERIALS AND METHODS

The designations AC 303,630 and CL 303,630 are used interchangeably by the company to refer to the active ingredient in this study. Company (trade) formulations are recognized by the name PIRATE.

The study was conducted using AC 303,630 labeled with ^{14}C in two separate ways: the 2-pyrrole carbon and uniformly in the phenyl ring. The pyrrole tracer had a specific activity of 56.5uCi/mg, was 97% radiopure by TLC and 97% chemically pure by HPLC. The phenyl label had a specific activity of 53.4uCi/mg, was 99% radiopure by TLC and 97% chemically pure by HPLC.

Solubility of CL 303,630 in deionized water and pH 4, pH 7, and pH 10 buffers at 25°C was previously measured by the company to be 0.12, 0.13, 0.14 and 0.12mg/L, respectively. For this study, commercial buffers of 0.05M KHP for pH 5.0, 0.05M potassium phosphate for pH 7.0 and 0.05M boric acid and potassium chloride for pH 9.0 were used. Triplicate samples at each pH and for each radiolabel were taken. Glassware was sterilized by autoclaving; buffer solutions, by passage through a 0.22 µm cellulose filter; aseptic conditions were maintained. Sample bottles were sealed and wrapped in foil to exclude light.

Stock solutions of the test compound in acetonitrile were made at concentrations of 0.027mg/mL and 0.030mg/mL for the 2-pyrrole and phenyl labels, respectively. For all samples an aliquot of stock solution was added to 150mL of the appropriate buffer. Final test solutions at each pH had a measured concentration of 0.07ppm for the 2-pyrrole label and 0.06ppm for the phenyl label. Each final test solution was used as a replicate for each sampling time. Three bottles each containing 150mL of sample for each pH and each radiolabel were removed from the constant temperature chamber at day 0, and at 7, 15, 21, and 30 days after dosing. Each solution was transferred and extracted with 30mL of ethyl acetate (EtAc). Triplicate aliquots were taken from both the EtAc and water phases to determine material balance of the radioactivity between the two phases. Likewise, for a second extraction. Combined EtAc extracts were concentrated. The concentrated residue/precipitate was dissolved in a methanol/EtAc solution. Original sample bottles were washed with 30mL of EtAc; aliquots were taken for radioassay.

Normal phase TLC of the methanol/EtAc extracts, with unlabeled CL 303,630 co-chromatographed as reference, was used to separate and help identify components. With silica-gel as substrate, the combined solvents hexanes:EtAc:acetic acid (100:50:0.75) were used to develop the chromatograms. Likewise, reverse phase TLC (Whatman KC₁₈F) was also used with the developer system methanol:water:acetic acid (150:50:0.5). The reference compound was visualized with UV light. Radioactive zones were visualized by autoradiography on X-ray film. Scrapings from the TLC plates were mixed with 4mL of water and 10mL of commercial scintillation cocktail. The resulting gelled mixture was then assayed by LSC.

In addition, for identification purposes only, two, two-dimensional TLC systems were used to test the zero and 30 day samples. Unlabeled reference CL 303,630 and the desbromo CL 303,094 were co-chromatographed in each instance.

HPLC analysis of the day zero and day 30 samples was performed on the concentrated methanol/EtAc solutions which were dissolved in acetonitrile:water (60:40). The mobile phase was acetonitrile:water:acetic acid (60:40:1). UV detection was at

260nm.

DATA SUMMARY

Autoradiograms of both normal and reverse phase TLC spots after thirty days showed one major spot which co-chromatographed with unlabeled CL 303,630 and contained virtually all radioactivity. Typical chromatograms (Fig. 1) and summary Tables V, VI and VII from the original submission are also included at the end of this record.

HPLC of the day zero and day 30 samples showed only one significant peak which chromatographed the same as reference AC 303,630; Fig. 2 is a sample chromatogram. Fig. 3 shows the relative positions of AC 303,630 and the desbromo AC 312,094, a possible degradate.

Overall, approximately 98% of the radioactivity partitioned from the aqueous phase into the EtAc. Except for one outlier in 90 replicates, more than 90% of the zero-time dose was recovered in every sample. These data are summarized in Tables II, III and IV of the original submission and are included as such at the end of this record.

DISCUSSION AND COMMENTS

At five appropriate intervals throughout a 30 day test period, in aqueous buffered solutions at pH's 5, 7 and 9 at 25°C, essentially all parent compound persisted. There was no evidence of trace hydrolysates. Triplicate samples were taken at all tested pH's and time intervals for each of two separately labeled ¹⁴C positions. TLC, HPLC and liquid scintillation counting techniques were used. Experimental conditions were well-controlled. The study conclusively shows that AC 303,630 is stable against hydrolysis (half-life indeterminately long in a 30 day test period).

The following comments and criticisms do not affect the acceptability of this study, but could affect the outcome in other cases.

1) Although not required, it is of potential value to know extremes of conditions (temperature, pH) under which this compound begins to hydrolyze or react, along with corresponding products and rates.

2) The systematically low ¹⁴C count rate in water before extraction on day zero should be explained or eliminated. This discrepancy, for which several possible reasons exist, is inconsequential here, but could be crucial in other instances.

3) Except for a token statement about radiocarbon counting

statistics, error analysis (limits) and resolution parameters are essentially absent throughout this study. There is also inconsistent and improper use of significant figures. A reviewer should not have to attempt to determine these from the data presented. Simple graphs, with error bars, could easily summarize all the radioactivity and concentration data. Nevertheless, inspection of the tabulated data and chromatograms does show them to be of good quality.

4) Because of the specific nature of AC 303,630, the study was unnecessarily exhaustive. Well-presented data and graphs, with statistical limits indicated, could have reduced the laboratory load and simplified review.

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Pages 18 through 22 are not included.

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- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
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- ☐ The product confidential statement of formula.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

STUDY 3

CHEM 129093 AC 303,630 PHOTODEGRADATION ON SOIL (§161-3)
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID (MRID) 42770242

Mangels, G. 1993. AC 303,630: Photodegradation on Soil.
Laboratory Project ID: ENV 93-014. Unpublished study performed
and submitted by American Cyanamid Company, Agricultural Research
Division, P.O. Box 400, Princeton, NJ.

REVIEWED BY: Alex Clem
TITLE: Environmental Scientist
ORG: EFGWB/EFED/OPP/OPPTS/EPA
TEL: 703-305-5991

SIGNATURE: Alex Clem

CONCLUSIONS

1) This study satisfies EPA data requirements for photodegradation on soil (§161-3).

2) At the end of a 30 day test period, approximately 75% of the parent compound remained, corresponding to an average half-life of approximately 75 ± 21 days for continuous photolysis with a UV filtered xenon-arc source. No photoproduct individually accounted for more than 10% (1.6 ppm) of the applied dose. Two identified products, the desethoxymethyl derivative (CL 303,268 in Table II) and 2-(4-chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-2-pyrrolidine-3-carbonitrile (CL 325,195 in Table II), each accounted for about 5% (0.8 ppm) of the original amount after 30 days. Six possible candidates for several unknown substances, none contributing more than about 3% (0.5 ppm), were eliminated. Thus the study identified about 85% of the dose present after 30 days, with several unidentified components probably exceeding 0.01 ppm. With total recoveries approaching 100%, volatile degradates, by difference, could not be formed at levels greater than a few percent. No further work on degradate chemistry or kinetics was stated.

MATERIALS AND METHODS

The designations AC 303,630 or CL 303,630 are used

interchangeably by the company to refer to the active ingredient in this study. Company (trade) formulations are recognized by the name PIRATE.

Radiolabeled AC 303,630. The study was conducted in side-by-side experiments using AC 303,630 labeled with ^{14}C either uniformly in the phenyl ring or on the 2-pyrrole carbon. The phenyl tracer had a specific activity of 53.4 $\mu\text{Ci}/\text{mg}$, was 97% radiopure by TLC and 97% chemically pure by HPLC. The pyrrole tracer had a specific activity of 56.5 $\mu\text{Ci}/\text{mg}$, was 97% radiopure by TLC and 97% chemically pure by HPLC.

Soil. Sassafras sandy loam obtained from the company Agricultural Research Center in Princeton, NJ was used. Characterization of this soil is given in Table I. The soil was sieved through a 2 mm mesh screen. Presumably the soil was air-dried.

Sample preparation and treatment. Precisely weighed, duplicate soil samples of about 5 g (presumed air-dry) were put in 5 cm diameter petri dishes, giving an average soil thickness of 1-2 mm. Stock solutions of the phenyl and pyrrole labeled compounds were made in acetonitrile at radioassayed concentrations of about 80 ppm. AC 303,630 was applied to the soil at a rate of 16 ppm by pipetting a 1 mL aliquot of stock solution onto the surface. Whether the surface was uniformly wetted was not stated. The solvent was allowed to evaporate from the samples, and petri dishes were covered (how they were covered was not stated). Control samples were wrapped in aluminum foil and placed in the dark in a constant temperature room at $25 \pm 1^\circ\text{C}$. The test samples to be irradiated were placed in a photolysis chamber also maintained at $25 \pm 1^\circ\text{C}$. Light from a xenon-arc lamp was filtered thru borosilicate glass to eliminate wavelengths below 290 nm, and was set at an intensity of $0.35 \text{ W}/\text{m}^2$ at 340 nm, which was said to be comparable to mid-autumn sunlight in Princeton, NJ. Samples were irradiated continuously with the spectrum of emitted light given in Figure 1.

Sampling schedule. Duplicate samples from each of the two labels were taken at 0 time and after 7, 14, 21, and 30 days of continuous radiation. Duplicate controls were assayed along with each set of irradiated samples, making a total of 18 samples in dishes for each label (time zero samples served as their own controls).

Extraction. Soil in each dish was extracted three separate times with 20 ml of 2% HCl/methanol by reciprocal shaking for 30 minutes, followed by 20 minutes of centrifugation. Supernatants were separately removed for subsequent radioassay.

Radioassay. Triplicate aliquots of the extracts were

radioassayed by LSC to help determine apportionment of radioactivity for a material balance. After extraction, soils were air-dried and thoroughly mixed. Five 0.250 g aliquots of soil were analyzed for residual radioactivity by automatic oxidative combustion followed by LSC.

Thin-layer chromatography. The three 2% HCl/methanol extracts were combined and neutralized with 50% NaOH. A 20 mL subsample was evaporated to a solid residue which was then taken up with 1 mL of water and extracted 3 times with ethyl acetate. These extracts were combined and concentrated to about 1 mL for assay by TLC. Aliquots (10 μ L) of the concentrates were co-chromatographed with standard (unlabeled) reference compounds (Table II) using two normal and one reverse phase system for the purpose of identification of components. Day 30 samples were additionally analyzed with a third normal phase TLC system. Scraped zones were quantitated by LSC of gel-cocktails. Unlabeled compounds were visualized with UV light, radiolabeled compounds by autoradiography.

High performance liquid chromatography. Several soil extracts were assayed by reverse phase HPLC to confirm that almost all the radioactivity was parent compound and to help confirm the identity of two minor metabolites, CB 303,268 and CL 325,195 (see Table II), which had been tentatively identified by TLC. Radiochromatograms were plotted and compared to the UV chromatograms of the extracts and reference compounds, respectively (Figures 7-9).

DATA SUMMARY

Side-by-side studies were conducted using AC 303,630 radiolabeled either in the phenyl or pyrrole ring. The test compounds were applied to the same type of sandy loam soil as used in the aerobic soil metabolism study at an actual experimental rate of 16 ppm, not the proposed field application rate of 0.40 lb/acre (0.44 kg/ha) or 0.2 ppm based on a 6 inch soil incorporation. Samples were irradiated continuously for up to 30 days with light from a xenon-arc source which was filtered to remove wavelengths shorter than 290 nm. Controls were kept in the dark. Temperature was maintained at 25 \pm 1°C.

Samples were assayed in duplicate at 0, 7, 14, 21 and 30 days of irradiation. Each soil sample was extracted three times with 2% HCl/methanol, which removed approximately 100% of the dose at time zero. At later times, the extracted dose decreased slowly to approximately 90% of the initial radioactivity for both labels. Within limits of precision, essentially all of the dose from the controls was extracted at all time intervals. The extracted soil was combusted to determine residual activity which ranged from about 0.1% to about 6% of the applied dose from both labels. Residual soil activity in the combusted controls ranged

from about 0.1% to about 1% of the applied.

The distribution of recovered radioactivity is summarized in Tables III and IV for the phenyl label and the pyrrole label, respectively. Between 95 and 105% of the phenyl and between 95 and 102% of the pyrrole label were recovered. With these high levels of recovery, volatile degradates could not have been formed at levels greater than a few percent.

Results of the TLC analyses are summarized in Tables V and VI for the phenyl and pyrrole labels, respectively. The Tables show that AC 303,630 was stable in the dark. The amount of parent in the extracts decreased with time from about 100% at time zero to about 82% after 30 days. The total recovered AC 303,630, as percent of applied dose, decreased from about 97% at time zero to approximately 75% after 30 days of irradiation for both labels. Typical autoradiograms are in Figures 2-5.

Plots of the logarithm of percent of parent tracer vs irradiation time are in Figure 6. For both labels, first order kinetics was approximated, and yielded half-lives of about 68±7 days and 82±20 days, respectively, for the phenyl and pyrrole labels undergoing continuous radiation. Data for least squares analysis and derived parameters are given in Tables VII and VIII. Assuming a combined half-life to be meaningful, an average half-life for the two labels is about 75±21 days for continuous irradiation.

SUMMARY AND COMMENTS

Under proper experimental conditions and with adequate controls and adequate material balance, AC 303,630 was found to photolyze on soil with an average half-life of approximately 75±21 days under continuous irradiation (see Data Summary above).

No photoproducts from either label individually accounted for more than 10% (1.6 ppm) of the applied dose. Two photoproducts, CL 303,268 and CL 325,195 (Table II), each contributed about 5% (0.8 ppm) of the dose after 30 days. Their identity was tentatively determined by TLC and confirmed by HPLC. Several unidentified radiolabeled components, none of which ever contributed more than 3% (0.5 ppm) of the applied dose, were detected and assayed. Control samples were stable for the duration of the study. The following six unlabeled standards (Table II) did not co-chromatograph with any of the unknown radiolabeled substances, and thus were eliminated as possible products: CL 303,793, CL 312,094, CL 322,116, CL 322,118, CL 325,008 and CL 325,157.

At the end of the 30 day test period, about 85% of the dose remaining had been identified: approximately 75% was parent plus the two compounds above, each at 5%. The reviewer infers that

the parent-like molecules which are being produced and appear to be accumulating, may be long-lived. Depending on results of other studies, further work on the chemistry and kinetics of products could be necessary before a definitive environmental fate assessment is made.

This soil photolysis study was well-conducted and well-presented. The following comments, criticisms and points of clarification do not affect the acceptability of the study or change conclusions, but should be considered when preparing future submissions.

- 1) More detail on the physical form and handling of the repurified, radioactive starting materials for the spiking solutions should have been given. Although it is implicit throughout the study that AC 303,630 has a low solubility in water, solubility is not given. Each study should stand on its own, and not assume prior, unreferenced knowledge of previous studies and lab procedures involving AC 303,630.
- 2) The computation of maximum concentrations of unidentified products (or any product) should be based on the actual weights of materials used in the experiment, not some hypothetical field use rate. Thus 3% of the 16 ppm (not 0.2 ppm based on a 6 inch soil incorporation) actually used is 0.5 ppm, which is certainly more than 0.01 ppm. On this basis, the study identifies about 85% of the dose present after 30 days, with several unknown components exceeding 0.01 ppm. However, identification of additional degradates is not likely to be crucial for environmental fate decisions.
- 3) It was preferable that this study were coordinated with the adsorption/desorption study, so that the sandy loam soil used would be the same, unless there is reason to the contrary.
- 4) Use of significant figures was inconsistent and sometimes improper.
- 5) Error limits based on actual counts should be included in the determination of standard concentrations and the concentrations of metabolites. Poisson (binomial) statistics should be used with these radioactivity measurements.
- 6) To avoid confusion, those columns of raw data for the soil marc (residue) which are headed as "aliquot" and "volume" should be indicated in mass (gram) units, not in the volume units used for the extracts listed above them.
- 7) Raw data, tables, figures, graphs, and chromatograms were very well-presented. The reviewer was pleased with the detailed and helpful inclusion of equations, formulas and least square (regression) parameters.

Page _____ is not included in this copy.

Pages 29 through 46 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
- ☐ A draft product label.
- ☐ The product confidential statement of formula.
- ☐ Information about a pending registration action.
- ☒ FIFRA registration data.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

STUDY 4

CHEM 129093 AC 303,630 AEROBIC SOIL METABOLISM (\$162-1)
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID (MRID) 42770243
Mangels, G. 1993. AC 303,630: Aerobic Soil Metabolism.
Laboratory Project ID: ENV 93-013. Unpublished study performed
and submitted by American Cyanamid Company, Agricultural Research
Division, P.O. Box 400, Princeton, NJ.

REVIEWED BY: Alex Clem
TITLE: Environmental Scientist
ORG: EFGWB/EFED/OPP/OPPTS/EPA
TEL: 703-305-5991

SIGNATURE: Alex Clem

CONCLUSIONS

- 1) This study satisfies EPA data requirements for aerobic soil metabolism (\$162-1).
- 2) At the end of a one year test period, approximately 82% of the parent compound remained, corresponding to a half-life of 1.4×10^3 days (3.8 years). The only metabolite ever found to be present at concentrations $>0.01\text{ppm}$ was identified as the desbromo derivative, and appeared to have accumulated gradually to approximately 8% (0.08ppm) of the total dose by the end of the study. Several unidentified substances, each of which accounted for less than 1% (0.01ppm) of the parent, were detected. Trapped $^{14}\text{CO}_2$ was insignificant. No further work on metabolite chemistry or kinetics was stated.

MATERIALS AND METHODS

The designations AC 303,630 or CL 303,630 are used interchangeably by the company to refer to the active ingredient in this study. Company (trade) formulations are recognized by the name PIRATE.

Radiolabeled AC 303,630. The study was conducted in side-by-side experiments using AC 303,630 labeled with ^{14}C either uniformly in the phenyl ring or on the 2-pyrrole carbon. The phenyl tracer had a specific activity of $53.4\mu\text{Ci}/\text{mg}$, was 99% radiopure by TLC and >97% chemically pure by HPLC. The pyrrole tracer had a specific activity of $56.5\mu\text{Ci}/\text{mg}$, was 98% radiopure by TLC and >97% chemically pure by HPLC.

Soil. Sassafras sandy loam obtained from a company agricultural research field in Princeton, NJ was used. Characterization of this soil is given in Table I. The soil was sieved through a 2mm mesh screen and air-dried to a moisture content of about 10%. During the experiment the final moisture content of the treated soil was maintained at 75% of 1/3 bar (11% by weight) by periodically weighing the samples and adding water as necessary.

Sample preparation and treatment. Precisely weighed, duplicate soil samples of about 100g (dry basis), were placed in 16oz wide-mouth glass jars and spiked with either 2.01mL of phenyl stock solution or 2.17mL of pyrrole stock solution. These volumes corresponded to $99\mu\text{g}$ of the test compound or a treatment rate which was stated (see comments) to be approximately 11b a.i./acre (1.1kg/ha). The spiking solutions were made with a water:acetone solvent ratio of 60:40 to help dissolve and disperse the test compound in the soil. The spiked soil was mixed by manually shaking the jars.

Jars were covered and connected to a water-saturated air flow system during incubation. Duplicate samples of each time point were connected in series such that the air exiting one replicate entered the second. Air leaving the second replicate was bubbled through ethylene glycol to trap volatile organics and then through 0.1N NaOH to trap CO_2 .

Sampling schedule. Traps for volatiles were sampled at appropriate intervals during the 12 month study. Duplicate soils were sampled at the time of dosing and after 1, 2 and 3 weeks, and after 1, 2, 3, 4, 6, 9 and 12 months of incubation.

Extraction. Soil samples were extracted once with water and four times with methanol. Mixtures were shaken for one hour and centrifuged for 30 minutes. Supernatants were brought to a known volume and radioassayed. The 6, 9 and 12 month soil samples were additionally and sequentially extracted with HCl in methanol/water and NaOH in methanol/water; as the radioactivity became more difficult to remove, additional extractions with aqueous NaOH were made.

Radioassay. Triplicate aliquots of the extracts were radioassayed by LSC. After extraction, soils were air-dried and analyzed for residual radioactivity by automatic oxidative

combustion followed by LSC.

Thin-layer chromatography. An intricate scheme of concentration of extracts and reextraction and dissolution of precipitates with acetone was used to prepare radioactive samples and subsamples for TLC. Two normal phase (three for the 12-month samples) and one reverse phase system were used with unlabeled AC 303,630 co-chromatographed. The unlabeled reference compounds listed in Table II were chromatographed to measure R_f values to help identify possible degradates. Unlabeled compounds were visualized with UV light, radiolabeled compounds by autoradiography.

High performance liquid chromatography. Several soil extracts were assayed by reverse phase HPLC to confirm that almost all the radioactivity was parent compound and to help confirm the identity of the minor desbromo metabolite, CL 312,094 (see Table II), which had been tentatively identified by TLC. HPLC analysis also showed that radiolabeled CL 303,268 was not present. Radiochromatograms were plotted and compared to the UV chromatograms of the extracts and reference compounds, respectively (Figures 5-7).

DATA SUMMARY

Suitably radiolabeled test compounds were applied to a sandy loam soil at an acceptable rate. A flow-through aeration system with traps for volatiles was used. The soil was incubated in the dark at 25±1°C; moisture content was maintained at 75% of 1/3bar. Soil samples were assayed in duplicate at dosing and after 1, 2 and 3 weeks and 1, 2, 3, 4, 6, 9 and 12 months of incubation.

A single water extraction only removed 0.4-0.6% of the dose throughout the study. Four methanol extractions removed at least 95% of soil radioactivity through the first 6 months. Methanol extracted about 92% at 9 months and about 90% at 12 months. More radical extractions of the 6, 9 and 12 month samples with HCL and NaOH additionally removed approximately 4% of the dose. Residual radioactivity in the soil after final combustion analysis increased from about 0.5% of the time zero dose, to about 3% at 4 months. After the acid and base extractions, residual soil radioactivity for the 6, 9 and 12 month samples was between 0.3 and 0.7% of the dose.

The distribution of recovered radioactivity is summarized in Table III for the phenyl label and Table IV for the pyrrole label (see comment 3 below). Between 95 and 104% of the phenyl dose and between 95 and 102% of the pyrrole dose was recovered. After one year, any volatiles which may have been trapped were barely above background.

Results of the TLC analysis are summarized in Table V for

the phenyl label and Table VI for the pyrrole. The total AC 303,630 recovered as percent of dose decreased from about 97% to about 82% after one year. Typical autoradiograms are in Figures 1-4. One metabolite, CL 312,094 (see Table II), accounted for a maximum of about 8% (0.08ppm) of the dose from each label. Several unidentified degradates, none of which ever contributed to more than 1% (0.01ppm) of the dose, were detected. HPLC analysis also indicated the presence of CL 312,094 and the absence of the possible degradate, CL 303,268 (Table II). HPLC radiochromatograms are in Figures 5-7.

Plots of the logarithm of percent of parent tracer vs time are in Figures 8 and 9. For both labels, first order kinetics was approximated, and yielded a half-life of about 1.4×10^3 days or 3.8 years. Least squares analysis data and derived parameters are given in Tables VII and VIII.

SUMMARY AND COMMENTS

AC 303,630 was incubated in the dark at 25°C in a sandy loam soil under moisture controlled and aerobic conditions for one year. Its first-order half-life was approximately 1400 days (3.8 years). The only identified metabolite, CL 312,094, accounted for as much as about 8% of the dose from either label. Of the several unidentified degradates, none accounted for more than 1% of the total doses. No further work on metabolite chemistry or kinetics was stated.

The one year study collaterally indicated that the metabolites which were detected appeared to be of low water extractability and potentially persistent. Depending on results of other studies, further work on metabolite chemistry and kinetics could be necessary before an environmental fate assessment is made.

This aerobic soil study was reasonably thorough and well-conducted. The following comments and criticisms do not affect the acceptability of the study or change conclusions, but should be considered when preparing future submissions.

1) More detail on the preparation of the water/acetone spiking solutions, and the physical form and handling of the repurified, radioactive starting materials should have been given. Although it is implicit throughout the study that AC 303,630 has a low solubility in water, solubility is not given. Each study should stand on its own, and not assume prior, unreferenced knowledge of previous studies and lab procedures involving AC 303,630.

2) The treatment rate for the experiment should be compared to the proposed field use rates which were not given in this study. By the reviewer's reckoning, 99µg per 100g of soil is

about 1ppm, which corresponds to about 2lb/acre (2.2kg/ha), not the stated 1lb/acre. (This calculation is based on a fairly standard assumption that an acre of soil six inches deep weighs two million pounds.) Through information from other submissions, the reviewer believes the proposed maximum field application rate is to be 0.4lb/acre. Nevertheless, the experimental rate chosen was appropriate for this chemical.

3) The principal material balances given in Appendix 4 were systematically high through the first 6 months, averaging about 108%. For months 9 and 12 the balance drops by about 5%. Was there an attempt to trace the causes for either of these occurrences. What is the justification for normalized values (recalculated according to the formula given in Appendices 1 and 2) given in Tables III and IV, and where did the missing material at 9 and 12 months go? Specific information was given on $^{14}\text{CO}_2$ trapped in NaOH, but no direct information on volatiles trapped in ethylene glycol was stated. Could this bear on material balance?

4) It was preferable that this study were coordinated with the adsorption/desorption study, so that the sandy loam soil used would be the same.

5) Use of significant figures was inconsistent and sometimes improper.

6) Error limits based on actual counts should be included in the determination of standard concentrations and the concentrations of metabolites. Poisson (binomial) statistics should be used with these radioactivity measurements.

7) Raw data, tables, figures, graphs, and chromatograms were very well presented. The reviewer was pleased with the detailed inclusion of equations and formulas.

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Pages 53 through 66 are not included.

The material not included contains the following type of information:

- ____ Identity of product inert ingredients.
- ____ Identity of product impurities.
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- ____ Identity of the source of product ingredients.
- ____ Sales or other commercial/financial information.
- ____ A draft product label.
- ____ The product confidential statement of formula.
- ____ Information about a pending registration action.
- ☒ FIFRA registration data.
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DATA EVALUATION RECORD

STUDY 5

CHEM 129093

AC 303,630 ADS/DES ON SOILS (§163-1)

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID (MRID) 42770244

Mangels, G. 1993. AC 303,630: Adsorption/Desorption on Soils. Laboratory Project ID: PD-M Volume 29-14. Unpublished study performed and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ.

REVIEWED BY: Alex Clem
TITLE: Environmental Scientist
ORG: EFGWB/EFED/OPP/OPPTS/EPA
TEL: 703-305-5991

SIGNATURE: 

CONCLUSIONS

- 1) This study is upgradeable and partially satisfies EPA data requirements for adsorption/desorption on soils (163-1).
- 2) In spite of shortcomings in the reporting of experimental procedures and calculation of results (see comments), AC 303,630, a compound of low aqueous solubility (0.12 ppm), was strongly bound to all test soils in this batch equilibrium study; therefore, it would not be expected to be mobile in soils. Tabulated adsorption parameters on p. 5.3 need to be recalculated by the registrant, but provide a valid estimate of magnitude and range.
- 3) For a complete and proper record, and before further studies on the compound are submitted to this section for review, the registrant should present the appropriate recalculated results, tables and figures, as well as provide their own material balance (see enumerated comments at end of DER).

MATERIALS AND METHODS

The designation AC 303,630 is used by the company to refer to the active ingredient in this study. Company (trade)

formulations are recognized by the name PIRATE. AC 303,630 labeled with ^{14}C on the 2-pyrrole carbon, with a specific activity of $135\mu\text{Ci/mg}$, 96% radiopure by TLC and 98% chemically pure by HPLC was used. Aqueous solubility of the material was previously measured by the company to be 0.12ppm at 25°C.

Characterization of the four appropriate test soils is given in Table II. All soils were sieved through a 2mm screen.

Adsorption. Four solutions of concentrations 0.11, 0.058, 0.011 and 0.0057ppm in 0.01M calcium chloride were used. Triplicate samples for each concentration and for each of the four soils were taken. Seven-hundred fifty milligram soil samples in 30mL aliquots of each of the four concentrations were equilibrated (two to three days with shaking were required) at 25°C in 50mL screw-capped centrifuge tubes. Samples were centrifuged, and aliquots of the supernatant were removed for analysis (see below). Most of the remaining supernatant was removed by pipette. Amounts of sample removed and remaining were determined gravimetrically by difference.

Desorption. Thirty mL of fresh 0.01M calcium chloride solution were added to the soil remaining in the centrifuge tubes after the adsorption procedure. After equilibration for three days, the same procedures were used as in the adsorption above.

Soil Extraction. The soil precipitate remaining from the desorption test was extracted by shaking with 25mL of methanol for one day. Extracts were treated and sampled as before.

Radioassay. Aliquots from all procedures above were radioassayed by LSC. Soils were air-dried, pulverized and analyzed for residual radioactivity by combustion to carbon dioxide followed by LSC.

Thin-Layer Chromatography. Aliquots from the high concentration adsorption and desorption solutions were extracted with ethyl acetate. These extracts and the methanol extracts from the soil were assayed using three one-dimensional TLC systems: 1) methylene chloride, 2) methylene chloride:hexanes (1:1) and 3) ethyl acetate:hexanes (2:8). Unlabeled parent compound was co-spotted in each case. Radiolabeled substances were visualized by autoradiography. It was not stated how unlabeled materials were visualized.

DATA SUMMARY

TLC autoradiograms showed one major spot which co-chromatographed with unlabeled AC 303,630. No information was provided for minor components. Typical chromatograms are given in Figs. 5-7.

Freundlich adsorption coefficients, K_f , and exponents, $1/n$, were calculated from the data using linear least squares analysis with R^2 given as the correlation coefficient. These values along with the average soil/water partition coefficients for adsorption and desorption, $K_d(ads)$ and $K_d(des)$, respectively, and K_{oc} , the adsorption coefficients normalized for organic carbon, were given as follows (caution--see conclusions above and comments below):

Soil	$K_d(ads)$	K_{oc}	K_f	$1/n$	R^2	$K_d(des)$
Arkansas loamy sand	37	13214	26.6	0.923	0.997	85
New Jersey sandy loam	120	14117	107	0.979	0.996	272
Wisconsin loam	168	12321	108	0.917	0.996	431
Indiana silt loam	190	18095	178	0.988	0.997	454
		14422-Avg.				

Logarithmic plots of X/M , where X is the mass of the compound adsorbed per unit mass M of soil, versus C_e , the aqueous phase equilibrium concentration, are given in Figs. 1-4.

DISCUSSION AND COMMENTS

This batch equilibrium study clearly demonstrated that AC 303,630 was tightly bound to the four test soils for four appropriate concentrations. Coupled with its low solubility, this compound is not expected to be mobile in soils.

Although the actual laboratory work and data collection are of good quality, the report was prepared carelessly and incompletely. It also assumed prior, unreferenced knowledge of previous lab procedures and studies involving AC 303,630. Each study should stand on its own or include adequate reference. The following comments and criticisms address specific issues in this study, but most have applicability for future submissions.

1) No material balance was given. By close and time consuming inspection and recalculation of some of the data, the reviewer found that the total radioactive material recovered systematically exceeded the dosed amount. Except for the three samples at the high concentration for the Arkansas soil, the other 45 samples had recoveries of over 100%, with several recoveries at the lower concentrations of 120 to 125%. This discrepancy was traced to the methanol extraction phase of the experiment. The density factor 0.79g/mL was correctly used in the computation of ppm from aliquots used in scintillation counting (see, for example, p. 27 of the original submission). However, it was inadvertently applied again through use of formula No. 5 on page 23 of the submission. This incorrect double use of the same factor results in a +21% error in the μg removed through extraction (see, for example, p. 13 of the original). When this error is propagated through other calculations it is of variable significance and changes some of

the reported results. Reviewer calculated material balance shows it to be acceptable when the correct calculation is made. Thus the compound was somewhat less strongly adsorbed than reported. Fortunately, because the compound was so strongly adsorbed, conclusions are the same.

2) No mention was made of how stock solutions were prepared and spiked.

3) No mention was made of how the soils were dried.

4) It was preferable that one of the soils used be the same as for the aerobic soil metabolism study.

5) No reference was made to other studies which gave information such as solubility and stability of the test compound for comparison with the conditions of the experiment.

6) Temperature control limits were not reported.

7) Use of significant figures was inconsistent and sometimes erroneous.

8) The reviewer was pleased with the inclusion of error limits and other statistical factors for some of the results. However, count rate statistics appear to be incorrectly calculated. They should be based on the actual number of counts collected and obey a binomial (or Poisson) distribution, not the normal distribution which appears to have been used. Consequently error limits given for concentrations are misleading. Error limits or error bars are not given in summarized results.

9) Raw data, tables, figures, graphs, and chromatograms were very well presented. The reviewer was pleased with the detailed inclusion of equations and formulas.

Page _____ is not included in this copy.

Pages 71 through 76 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
- ☐ A draft product label.
- ☐ The product confidential statement of formula.
- ☐ Information about a pending registration action.
- ☒ FIFRA registration data.
- ☐ The document is a duplicate of page(s) _____.
- ☐ The document is not responsive to the request.

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DATA EVALUATION RECORD

STUDY 6

CHEM 129093 CL 303,630 (AC 303,630) Accumulation in Fish (\$165-4)

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID (MRID) 42770245

Zulalian, Jack. 1993. CL 303,630: Uptake, Depuration, Bioconcentration and Metabolism of Carbon-14 CL 303,630 in Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Test Conditions. Laboratory Project ID: American Cyanamid No. MET 93-002, ABC Laboratories No. 39603-2. Unpublished study performed by American Cyanamid Company, Ag. Res. Div., Box 400, Princeton, NJ, and ABC Laboratories Inc., Aquatic Toxicology Programs Div., 7200 East ABC Lane, Columbia, MO. Submitted by American Cyanamid Company, Agricultural Research Division, Box 400, Princeton, NJ.

REVIEWED BY: Alex Clem
TITLE: Environmental Scientist
ORG: EFGWB/EFED/OPP/OPPTS/EPA
TEL: 703-305-5991

SIGNATURE: Alex Clem

CONCLUSIONS

1) This study satisfies EPA data requirements for accumulation in fish (\$165-4).

2) In spite of shortcomings in the study and in the reporting of experimental results (see comments), essential conclusions have been gained from the study. However, for a complete and accurate master record, and before further studies are submitted to this section for review, the registrant should primarily address the issues indicated by items 1, 2 and 3 in the comments section at the end of the DER. No additional lab work should be necessary.

3) Bluegill sunfish were continuously exposed to a nominal concentration of CL 303,630 (two separate ¹⁴C radiolabels) of 1 ppb for 33 days in a flow-through proportional diluter system.

A 21 day depuration period followed. Of the total radiolabeled CL 303,630 residues after 33 days, a reviewer calculated average of approximately 80% (3400X) accumulated in the nonedible tissues, 20% (830X) in the edible, but these were depurated with a $T(1/2)$ of roughly 4 days. After the 21 day depuration, 97% of total residues in all tested parts of the fish had been eliminated. One major and two minor metabolites were identified and quantified in aquaria water. The same major identified metabolite, the desbromo derivative CL 312,094 (see Table of Compounds), was present in the fish tissues and the aquaria waters, and accounted for up to 96% of the residues in fish tissues.

MATERIALS AND METHODS

The designation CL 303,630 is used by the company to refer to the active ingredient in this study. In other studies the designations AC 303,630 or CL 303,630 or both have been used. Company (trade) formulations are recognized by the name PIRATE.

Radiolabeled CL 303,630. The study was conducted in side-by-side experiments using CL 303,630 labeled with ^{14}C either uniformly in the phenyl ring or on the 2-pyrrole carbon. The phenyl tracer had a specific activity of $53.4 \mu\text{Ci}/\text{mg}$, was 99% radiopure by TLC and 97% chemically pure by HPLC. The pyrrole tracer had a specific activity of $56.5 \mu\text{Ci}/\text{mg}$, was 98% radiopure by TLC and 97% chemically pure by HPLC.

Stock solutions. Stock solutions were made in acetone and stored in a freezer until needed. Verification of purity and radioactivity (concentration) was made by HPLC and LSC, respectively. These solutions were added as necessary to the toxicant diluter mixing cell and injection system to maintain a nominal exposure concentration of around 1 ppb, which had been previously determined to be $1/10$ the LC_{50} value.

Water. All cultural and dilution water was from an uncontaminated deep well source. Characteristics are summarized in Table I. Water quality was maintained and measured during the experiment (Tables XVIII and XIX).

Test Fish. Six hundred bluegill sunfish (*Lepomis machrochirus*) estimated to be less than one year old were held in culture tanks according to standard techniques. Daily records were kept. Representative initial mean weight was $6 \pm 2 \text{ g}$ and mean standard length was $57 \pm 6 \text{ mm}$. At the end of depuration, control fish had a mean weight of $14 \pm 4 \text{ g}$ and mean standard length of $72 \pm 7 \text{ mm}$.

Test System. A flow-through proportional diluter system was used for intermittent introduction of CL 303,630 and dilution water into 100 liter glass test aquaria which were filled to

70 liters. Five aquaria were used: four treated aquaria (one for radioanalysis totals and one for metabolite determinations for each of the two radiolabels) and one control aquarium which received an amount of acetone (0.10 mL) equivalent to that delivered to the treated tanks. Aerated well water (Table I) was delivered to the aquaria at a rate of about 300 ml/min, which is equivalent to filling the tanks 6-7 times a day. Ample consideration was given to conditioning of aquaria and fish before the start of the experiment. To begin the test, the five aquaria each received 120 fish and were kept under constant aeration and continuous flow. Test fish appeared in good health throughout the study with no natural mortality.

Test Procedure--Uptake Phase. Water and fish were sampled during the uptake period according to the schedule in Table III. Fillet (edible) and viscera (nonedible) portions were sectioned as in Figure 1. Fish and water samples were immediately frozen and stored for the analysis procedures below.

Test Procedure--Depuration Phase. Based on three combusted whole fish samples on day 28, the uptake phase was extended to 33 days. On day 33, uptake was terminated and the exposed aquaria were purged and replenished with flowing, uncontaminated well water for 21 days. Water and fish were sampled according to Table III. Water and fish samples were handled and frozen as before for subsequent radioanalysis.

Radioanalysis and Extraction of Water Samples. About one liter of water had been removed from each aquarium on each sampling day. An additional liter of had been collected on days 21 and 33 of uptake and day 21 of depuration for metabolite characterization. Water samples of 530 mL were used. Concentrations of pre-extraction radioactivity, calculated as CL 303,630 in water, were determined in triplicate by LSC using 10 mL aliquots. Fortified control samples were also tested. Solid phase extraction with a C18 cartridge activated with acetonitrile was then used. The remaining 500 mL water samples were acidified with 40 mL of acetic acid, put on the cartridge and eluted with 14 mL of acetonitrile. The eluate was adjusted to 15 mL with acetonitrile. Aliquots of 0.5 mL were removed and analyzed by LSC to determine the extraction of total ¹⁴C-residues. Remaining eluant was concentrated and analyzed by LSC to determine radioactivity prior to injection for HPLC and fraction collection. The volume of post-extracted water samples was measured with a 1000 mL glass graduated cylinder, triplicate 10 mL aliquots were taken and analyzed by LSC to determine the amount of unextracted radioactive residues.

HPLC Analysis of Water Samples. All water samples and diluter stock solution samples from uptake were analyzed by HPLC. Injections of a CL 303,630 "7-component" standard bracketed the injections of the uptake samples to determine retention time of

each component. Bands of radioactivity in the fractions (detected by LSC) were compared with the standard reference to determine percentages of each extracted component. Based upon these percentages and previously determined total extracted radioactivity, the concentration of each component was calculated.

Radioanalysis of Fish. Fish had been sampled by the schedule in Table III. On each of these days three fish from each tank were collected. Control and treated fish were dissected into edible and nonedible parts (Figure 1). Three additional fish from each tank were pooled into control and treated samples for whole fish analysis. Also as indicated in Table III, additional fish were collected for metabolite analysis. For radioassay, individual frozen samples were homogenized with dry ice in a grinder, allowed to sublime and thaw under ambient bench top conditions, and weighed. Triplicate samples of the pooled homogenates were then combusted and analyzed automatically by LSC. Levels of radioactivity were reported as $\mu\text{g/kg}$ of CL 303,630 derived residues. NIST certified standard labeled benzoic acid was used to determine LSC recoveries. Spiked control tissue was tested and counting efficiencies determined. Minimum quantifiable limits for counting sensitivity were determined for each set of samples.

Extraction of Fish Tissues. Whole fish, fillet and viscera from day 21 and day 33 uptake were analyzed for the identification and quantitation of components in the residues. Fish samples were homogenized as before. The flow diagram for extraction and analysis of tissues is given in Figure 11.

The post-extracted solids (PES in the diagram) from the viscera collected at day 33 were incubated overnight with pepsin in HCL to release "bound" residues. The enzymatically digested tissues were extracted with trichloroacetic acid; aliquots of extract were assayed by LSC. The remaining insoluble solid was dried, aliquots weighed, combusted and quantitated by LSC. The trichloroacetic acid soluble fraction was extracted with methylene chloride. The radioactivity recovered in the extract and that remaining in the aqueous phase was analyzed by LSC. Solid phase extraction/clean-up was done on C18 cartridges and was followed by HPLC/ ^{14}C analysis. Spiked control PES viscera samples were also incubated with pepsin to assess the stability of CL 303,630 to the digestion procedure.

The remaining extracts in Figure 11 from the day 33 viscera samples were concentrated and analyzed in similar fashion, with appropriate columns and solvent combinations. In this manner, percent recovery and separation into components could be assessed. Two components were isolated and fractions were submitted for mass spectral analysis. A test was also made to determine if any volatilization loss occurred during sample

rotary evaporation procedures which were used for some of the concentration steps.

Thin Layer Chromatography. Two one-dimensional and one two-dimensional TLC systems were used to separate and tentatively identify the components in the extracts of fish tissues and aquaria water. Individual reference compounds were co-chromatographed with the test samples. Radiometabolites were detected by autoradiography, unlabeled reference compounds were visualized with UV light. LSC of radioactive zones was used to quantitate separated components.

Reference Compounds. Unlabeled CL 303,630 and each reference compound (see Table of Compounds) had their chemical structure verified by proton NMR or mass spectral analysis. A solution of each reference compound was used for determining TLC and HPLC properties and for identification and monitoring purposes.

DATA SUMMARY

Bluegill sunfish were continuously exposed to a nominal concentration of CL 303,630 (two separate ¹⁴C radiolabels) of 1 ppb (1/10 of LC₅₀) for 33 days in a flow-through proportional diluter system. A 21 day depuration period followed. Both fish and water were sampled at appropriate intervals. Proper controls and conditions were maintained and monitored throughout.

The registrant reported steady-state concentrations were reached after 33 days. Highest observed concentrations of total residues were 3700 ppb in the viscera, 2200 ppb in whole fish and 980 ppb in the fillet. Corresponding bioconcentration factors, calculated using the running means of aquaria water concentrations, were 3900X, 2300X and 1200X, respectively. After the 21 day depuration, a tight average of 97% of residues in all parts of the fish had been eliminated.

HPLC/¹⁴C analysis of aquaria water showed that four components were present and tentatively identified them in approximately the following amounts: parent CL 303,630--90%, CL 312,094--40%, CL 325,195--7%, CL 303,793--2%. This profile of extracted radioactivity was fairly constant from day 7 to day 33 of uptake. Parent and the major metabolite were isolated from the pyrrole-labeled water and identified by GC/MS.

Of the total residues from either radiolabel in all fish tissues, more than 95% were extracted. HPLC/¹⁴C analysis indicated that parent CL 303,630 accounted for only 2-3% (10-85 ppb); the major metabolite, the desbromo compound CL 312,094, accounted for 89-96% (585-3352 ppb) and minor polar, but unidentified metabolites accounted for 2-4% (11-140 ppb) of these

tissue residues. The parent and major metabolite were isolated and identified by LC/MS and GC/MS.

The non-extracted fish viscera residues (97-122 ppb) were hydrolyzed with pepsin/0.1N HCL to release 58% of the residue (55-71 ppb) as trichloroacetic acid-soluble products. These were also characterized by HPLC/¹⁴C, but only as polar metabolites "resembling" those minor components detected in the extracts of fish tissues above. Less than 3% of parent or the major metabolite above were detected in the hydrolysis products.

The Dow BIOFAC computer program was used to estimate kinetic and bioconcentration factors (BCF) by fitting the actual data for each radiolabel and for each fish tissue type. The parameters for whole fish (fillet + viscera), were taken by the reviewer and averaged over both labels. Results are as follows (caution--see item 1 in comment section below):

Uptake Rate Constant: 380 ± 30 $\mu\text{g}/\text{kg fish}/\mu\text{g}/\text{L water}/\text{day}$
Depuration Rate Constant: 0.18 ± 0.01 (fractional loss per day)
T(1/2) for Depuration: 3.9 ± 0.3 days
Bioconcentration Factor: 2100 ± 200
T(90%) to Steady State: 12.5 ± 0.9 day

COMMENTS

1) The reviewer is not convinced that steady-state had been reached after 33 days of uptake as is evidenced by several large increases in concentration (in one case over 50%) around day 33. Values for the bioconcentration and kinetic factors given by the Dow BIOFAC program would, of course, be different if steady-state had not been attained. Furthermore, it is apparent that the submitted computer generated curves were biased against continued uptake. It seems likely, therefore, that some unexplained source of error was present or that steady-state had not been attained. Perhaps some follow-up would have been appropriate. Any comments or explanations?

2) Merging of the data and results from ABC Labs with the tissue metabolite results from American Cyanamid was poorly correlated and poorly verified. Consequently, important information and numbers given by the study author in the opening summary and ending conclusions of the original submission do not jibe with results given by ABC Labs within the body of their study and in their data tables. Corrections are in order.

- 3) Conclusions by ABC Labs on their aquaria water analysis do not agree with the data in Tables XVI and XVII, with the chromatograms given in Figure 13, and with the detailed information given on pps. 115-116 of the submission. It is also apparent that the methods used by ABC Labs were more definitive than those of American Cyanamide. Please reconcile.
- 4) To avoid confusion the registrant should use the same name designation for the subject chemical in all studies. In various studies either AC 303,630 or CL 303,630 or both have been used.
- 5) Several section references in the merged study are misdirected.
- 6) It is the duty of the study author or his editors, not the reviewer, to ferret out and reconcile discrepancies such as those listed above.
- 7) Why is the relative concentration of degradates in aquaria waters so high?
- 8) Some minor extractable fish tissue degradates in excess of 50 ppb were not identified. However, additional information on these is not likely to be necessary for environmental fate decisions.
- 9) The study assumes prior knowledge of chemical specific information such as aqueous solubility and stability. Each study should stand on its own or provide adequate reference.
- 10) Some samples were stored frozen for about nine months. Although storage stability data was not given, related results in this study and in other studies known to the reviewer indicate that any effects should be inconsequential.
- 11) Good work on the discovery that CL 312,094 codistilled with methanol/water during the concentration step using rotary evaporation.
- 12) Raw data, tables, figures, etc., were well-presented. The inclusion of flow diagrams, equations, formulas and estimated kinetic parameters were very helpful.
- 13) The reviewer was impressed with the professionalism and high quality of the work given in the experimental sections, especially that of ABC Labs. What was obviously an outstanding laboratory effort was marred by a disorganized report of the results, which inadvertently omitted or did not accurately transfer some of the findings. The report seemed to emphasize form rather than substance and logic.

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Pages 85 through 104 are not included.

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