DATA EVALUATION RECORD

STUDY 1

CHEM 069008 Pyrethrin 1 §162-1
CAS No. 121-21-1
FORMULATION–00–ACTIVE INGREDIENT

STUDY ID 43499803
Robinson, R. A. 1994. Aerobic soil metabolism of [^{14}C]Pyrethrin 1. XBL Study No. XBL93061. XBL Report No. RPT00204. Unpublished study performed by XenoBiotic Laboratories, Inc., Plainsboro, NJ; and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C.

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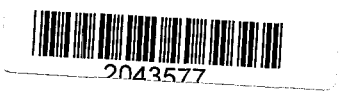
CONCLUSIONS

1. This study is considered acceptable and fulfills the aerobic soil metabolism (§162-1) data requirement. The study provides scientifically valid data showing that the aerobic degradation of cyclopropane ring-labeled [1-^{14}C]pyrethrin in sandy loam soil at nominal concentration of 1 ppm had biphasic character; the respective calculated first and second half-lives were 3.2 days (r^2 = 0.89; 0-14 days data) and 23.5 days (r^2 = 0.98; 14-59 day data). The overall half-life was 9.5 days (r^2 = 0.77). The major unidentified degradate, Reg-3, was a maximum of 29.7% at 3



days posttreatment and the minor degradate, chrysanthemic acid, was a maximum of 4.0% at 3 days posttreatment. The minor unidentified degradates Met-A, Met-B, Reg-1, Met-C, Reg-2, and Reg-4 were each present at less than 7.5% (0.08 ppm; single replicate) of the applied radioactivity (APR). Non-extractable residues accounted for an average maximum of 40.0% of APR at 30 days posttreatment. From this, 5.9% of APR was humin, 11.7% of the applied was fulvic acid, and 9.5% of APR was humic acid. Evolved $^{14}CO_2$ accounted for a maximum of 43.4% at 181 days. Material balance ranged from 82.7-101.1% of APR.

2. The following minor problems problems were found in the study. These problems are related to the guideline requirement for pyrethrin I aerobic soil metabolism study:

- Microbial viability of the test soil was not confirmed; since the rate of degradation of pyrethrin was relatively fast, it is presumed that the soil was viable.
- The analytical method detection limits were not reported, additionally it appears that a validation of the analytical methodology for the determination of total pyrethrins in soil was not conducted.
- Despite the fact that the bound residues were substantial, only one extraction was performed, and it is not known if additional extractions could have extracted additional material.

ABSTRACT
Metabolism - Aerobic Soil

Cyclopropane ring-labeled [1-^{14}C]pyrethrin, at a nominal application rate of 1 ppm, degraded with a registrant-calculated half-life of 9.5 days ($r^2 = 0.77$) in sandy loam soil adjusted to 75% of 0.33 bar moisture content and incubated in the dark at 25±1°C for up to 181 days. The degradation of pyrethrin was biphasic; half-lives were 3.2 days (0-14 day data) and 23.5 days ($r^2 = 0.98$; 14-59 day data). All data, designated as percentages of the applied radioactivity, represent percentages of the nominal application. Data are the means of duplicates, unless otherwise noted.

Based on HPLC analysis, the parent compound was initially 85.4% (0.85 ppm; reviewer-calculated), was 48.2% (0.48 ppm) at 2 days, was 15.6% (0.16 ppm) at 3 days, and was last observed at 1.03% (0.01 ppm; single replicate) at 59 days.

The major unidentified, region Reg-3, was initially (time 0) 4.6% (0.05 ppm) of the applied radioactivity, was a maximum of 29.7% (0.30 ppm) at 3 days, and decreased to 3.0% (0.03 ppm) by 59 days. This region appears to consist of a band of multiple peaks. On Day 3, Rep. A, two distinct peaks (28, and 31 minutes) were observed, but they were integrated along with the entire region (Reg. 3). The Rep. B shows only one of those peaks (31 min.).

The minor degradate, chrysanthemic acid (designated Met-B), and the minor unidentified degradates, Met-A, Met-B, Reg-1, Met-C, Reg-2, and Reg-4, were each present at <7.5% (<0.08 ppm; single replicate) of the applied radioactivity from 0 to 59 days posttreatment.

Nonextractable [^{14}C]residues accounted for a maximum of 40.0% (0.40 ppm) of the applied at 30 days posttreatment. Based on organic matter fractionation analysis, 5.9% of the applied radioactivity was humin, 11.7% was fulvic acid, and 9.5% was humic acid.

Evolved $^{14}CO_2$ initially (time 0) accounted for 0.32% of the applied at 0.5 days posttreatment and was a maximum of 43.4% (0.43 ppm) at 181 days.

MATERIALS AND METHODS

Samples of moist, sieved (2 mm) sandy loam soil (68% sand, 18% silt, 14% clay, pH 6.4, 3.7% o.m., CEC 18.0 meq/100 g; Table III, p. 38) from Grand Forks County, North Dakota, were weighed (50 g, dry weight) into biometer flasks and treated with cyclopropane ring-labeled [1-^{14}C]pyrethrin 1 {2-methyl-4-oxo-3-(2,4-pentadienyl)-2-cyclopenten-1-yl ester; specific activity 11.5 mCi/mmol, radiochemical purity 96.08%; Amersham Life Sciences, Buckinghamshire, England; Table I, p. 36; diagram presented on p. 13}, dissolved in acetonitrile, at a nominal application rate of 1 ppm (p. 16). Immediately following treatment, the soil was adjusted to 75% of 0.33 bar moisture content, and 1 N KOH (10 mL) was placed in the sidearm of the flask to trap $^{14}CO_2$. The test systems were sealed and incubated in the dark at 25 ± 1°C (p. 17; diagram not presented). Additional beakers of untreated control soils were prepared using the same method. Bulk samples for metabolite identification were fortified at 10 ppm using the same method. Duplicate flasks of treated soil (1 ppm) were collected for analysis at 0, 0.5, 1, 2, 3, 7, 14, 30, 59, 121, and 181 days posttreatment; bulk samples (10 ppm) were collected for analysis at 14 and 121 days posttreatment (Table IV, p. 39). Additional samples (1 and 10 ppm fortifications) and untreated controls were collected and placed in frozen storage. Volatile traps were analyzed and replaced at each sampling interval.

At each sampling interval, soil samples were extracted by blending with acetonitrile:1% aqueous HCl (4:1, v:v) for 2 minutes (p. 18). The samples were vacuum filtered (membrane not specified); the filtrate was collected and partitioned twice with methylene chloride. Following phase separation, aliquots of the aqueous (designated Aqueous-1) and organic (designated CH_3CN/CH_2Cl_2) phases were analyzed for total radioactivity by LSC; the limit of detection was not reported. Aliquots of the organic phase (CH_3CN/CH_2Cl_2) were analyzed by reverse-phase HPLC operated using the following conditions (p. 20):

Column	Ultracarb 5 ODS, 4.6 x 250 mm, 5 µm (Phenomenex #50675 and #31775)	
Injection volume	10-100 µL	
Detectors	UV (254 nm); radioactive flow monitor	
Mobile phase	acetonitrile:0.025 M KH_2PO_4/H_3PO_4 (approximately pH 4)	water:acetonitrile:0.1 M NH_4OAc
Mobile phase gradient	20:80 to 80:20, v:v (days 0 to 121)	0:20:80 to 0:80:20 to 20:80:0 to 0:100:0 to 100:0:0, v:v:v (day 181)
Retention time, parent	approximately 35.2-35.6 minutes	

Selected samples (not specified) were co-chromatographed with nonradiolabeled reference standards of the parent and the potential degradate chrysanthemic acid, and the radiolabeled reference standard [^{14}C]chrysanthemic acid (p. 20). Eluent fractions were collected at 1-minute intervals and analyzed by LSC (p. 23). Post-extracted soil samples were analyzed for total radioactivity by LSC following combustion (p. 18); combustion efficiency was not reported.

Selected (day 30) post-extracted (acetonitrile:1% aqueous HCl) soil samples were extracted by refluxing with 0.25 N HCl for 1 hour, cooled, and filtered (membrane not specified; p. 19). The filtrate was partitioned twice with ethyl acetate. Following phase separation, the aqueous (designated AQ-2)and organic (designated EtOAc-1) phases were analyzed by LSC and HPLC as previously described (p. 33).

To confirm the identity of the parent compound and the potential degradate chrysanthemic acid, the organic phase extracts (CH_3CN/CH_2Cl_2) were analyzed by one-dimensional TLC on silica gel plates developed with either cyclohexane saturated with formic acid:ether (3:2, v:v) OR methylene chloride:ethyl acetate (3:1, v:v; p. 23). Radiolabeled residues were visualized by radioimage analysis. Samples were co-chromatographed with nonradiolabeled reference standards of the parent and the potential degradate chrysanthemic acid, which were visualized by UV light (254 nm; p. 24). Further, samples were derivatized with either *N,O*-bis(trimethylsilyl)-trifluoroacetamide, –methyl-*N*-(*tert*-butyl-dimethylsilyl)-trifluoroacetamide, Deriva-Sil®, or by methylating with diethyl ether diazomethane (p. 26); aliquots of derivatized and nonderivatized samples were analyzed by GC/MS using the following operating conditions (p. 25):

Column: Rtx-1, 15 m x 0.25 mm i.d., 0. 25 µm (Restek)
Temperature: 70°C for 5 minutes, increase to 300°C at 15°C/minute, hold at 300°C for 2 minutes
Injector Temperature: 230°C (splitless)
Detector: MS in positive ion electron impact mode (temperature not specified)
Carrier Gas: Helium (flow rate not specified)
Scan Range: 35-600 m/z

To determine organic matter fractionation, the refluxed (HCl) sediment was extracted by shaking with 0.5 N NaOH (approximately 100 mL) for 24 hours at approximately 20°C and filtered (membrane not specified). The filtrate (designated Hydrolysate-2) was collected and an aliquot was analyzed by LSC. The filtrate (Hydrolysate-2) was acidified (HCl), centrifuged, and the supernatant was decanted. The precipitate (humic acid) was dissolved in 0.5 N NaOH and analyzed by LSC. An aliquot of the supernatant (fulvic acid) was analyzed by LSC; an aliquot was also partitioned twice with an equal volume of ethyl acetate. Following phase separation, duplicate aliquots of the organic and aqueous phase extracts were analyzed by LSC. The post-extracted (NaOH) sediment (humin) and the filter membrane were air dried and analyzed by LSC following combustion.

Volatile trapping solutions were collected at each sampling interval; duplicate aliquots were analyzed for total radioactivity by LSC (p. 17). $^{14}CO_2$ was confirmed by barium precipitation (p. 18).

RESULTS/DISCUSSION

Cyclopropane ring-labeled [1-^{14}C]pyrethrin (radiochemical purity 96.08%), at a nominal application rate of 1 ppm, degraded with a registrant-calculated half-life of 3.2 days (r^2 = 0.89; 0-14 days data) in sandy loam soil adjusted to 75% of 0.33 bar moisture content and incubated in the dark at 25 ± 1°C for up to 181 days posttreatment (Table VII, p. 42). However, the degradation of pyrethrin was biphasic; the first half-life occurred at 1.3 days (r^2 = 0.87; 0-3 day data). All data, designated as percentages of the applied radioactivity, represent percentages of the nominal application. Data are the means of duplicates, unless otherwise noted.

Based on HPLC analysis, the parent compound was initially present at 85.4% (0.85 ppm), was 65.4% (0.65 ppm) by 1 day posttreatment, was 48.2% (0.48 ppm) at 2 days and 15.6% (0.16 ppm) at 3 days, decreased to 1.03% (0.01 ppm; single replicate) by 59 days (Table VII, p. 42).

The major unidentified degradate, Reg-3, was initially (time 0) detected at 4.6% (0.05 ppm) of the applied radioactivity, increased to 10.9% (0.11 ppm) by 0.5 days, was a maximum of 29.7% (0.30 ppm) at 3 days, and decreased to 3.0% (0.03 ppm) by 59 days, the last sampling interval data were reported (Tables VII and VIII, pp. 42 and 43). This region appears to consist of a band of multiple peaks. On Day 3, Rep. A, two distinct peaks (28, and 31 minutes) were observed, but they were integrated along with the entire region (Reg. 3). The Rep. B shows only one of those peaks (31 min.).

The minor degradate, chrysanthemic acid(designated Met-D), was initially (time 0) present at 1.1% (0.01 ppm), was a maximum of 4.0% (0.04%) at 3 days posttreatment, and was 0.27% (0.003 ppm) at 59 days. The minor unidentified degradates Met-A, Met-B, Reg-1, Met-C, Reg-2, and Reg-4 were each present at less than 7.5% (0.08 ppm; single replicate) of the applied radioactivity from 0 to 59 days posttreatment.

Nonextractable [^{14}C]residues accounted for a maximum average of 40.0% (0.40 ppm) of the applied radioactivity at 30 days posttreatment (Table V, p. 40). Based on organic matter fractionation analysis, 5.9% of the applied radioactivity was humin, 11.7% of the applied was fulvic acid, and 9.5% of the applied was humic acid (p. 33; Appendix G, pp. 162-163).

Evolved $^{14}CO_2$ initially accounted for 0.32% of the applied radioactivity at 0.5 days posttreatment and was a maximum of 43.4% (0.43 ppm) at 181 days (Table V, p. 40).

Material balances (based on LSC analysis of individual replicates) were 82.7-101.1% of the applied radioactivity (Table VI, p. 41).

COMMENTS

1. Microbial viability of the test soil was not confirmed. Subdivision §N Guidelines require that the samples be analyzed for microbial activity.

2. The samples were adjusted to 75% of 0.33 bar at the initiation of the study period (p. 16); however, the reviewer could not confirm whether the moisture content was maintained throughout the study. Clarification from the registrant may be required. According to the protocol, the moisture of the soil would be verified at specific intervals.

3. A validation of analytical methodology for the determination of total pyrethrins in soil was not conducted.

4. The degradation rate of the parent compound was biphasic; the respective reviewer-calculated first and second half-lives were 3.2 days ($r^2 = 0.89$; 0-14 days data) and 23.5 days ($r^2 = 0.98$; 14-59 day data).

5. Limits of quantitation and detection were not reported for HPLC and LSC analysis. It is necessary that both limits of quantitation and detection be reported to allow the reviewer to evaluate the adequacy of the test method for the determination of the parent compound and its degradates.

6. Data were not reported for the parent compound in the aqueous phase extracts; however, the study author stated that, in the aqueous fraction, total radiolabeled residues averaged only 0.07-2.52% of the applied radioactivity during the study period (p. 28; Table VI, p. 41).

7. Organosoluble residues were only identified up to 59 days posttreatment; however, total radioactivity in the organic and aqueous extracts, $^{14}CO_2$, and post-extracted soil was reported at 121 and 181 days (Table V, p. 40). The study author stated that no attempt was made to identify residues at 121 and 180 days posttreatment since less than 1% of the parent compound was present at 59 days posttreatment, and because of the "apparent low contribution" of several (number not specified) minor degradates present in the organic extracts (p. 29).

8. The residue concentrations (in ppm) of the radiolabeled residues and volatiles were calculated by the reviewer based on the actual application rate (1 ppm) and the percentages of the recovered radioactivity.

9. Aliquots of organic phase extracts (CH_3CN/CH_2Cl_2) derivatized and nonderivatized samples were analyzed by GC/RAM (p. 25).

10. The maximum label rate of the test substance was not reported.

11. The soil series name of the test soil was not reported.

12. The chemical name of pyrethrin 1 was not reported. However, the reviewer noted that the chemical name is reported as 2-methyl-4-oxo-3-(2,4-pentadienyl)-2-cyclopenten-1-yl ester in the USEPA/OPP Pesticide Related Database.

13. In a preliminary study phase, 50 g (dry weight basis) of soil were treated with test substance at 1 ppm treatment rate, and adjusted to ~75% of field moisture capacity. Each sample had a polyurethane plug to trap neutral volatiles, and a trap with 10 mL 1N KOH. Sampling was performed in duplicate at 0, 3, and 7 days, and a single sample at 1 day. The results indicated that pyrethrin would degrade very rapidly (estimated half-life 2.9 days). Based on these results, additional sampling intervals were added to the definitive study.

14. Samples were treated at a high rate of 10 ppm for the purpose of metabolites identification, but instead, representative samples were harvested for material balance and product profiling of extractable residues.

ATTACHMENT 1
Tables and Figures

Pyrethrins review

Page ____ is not included in this copy.

Pages __9__ through __17__ are not included in this copy.

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.

__X__ FIFRA registration data.

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

_____ The document is not responsive to the request.

_____ Internal deliberative information.

_____ Attorney-Client work product.

_____ Claimed Confidential by submitter upon submission to the Agency.

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.

ATTACHMENT 2
Excel Workbook

MRID 43499803

Days posttreatment	% Parent	Ln (% parent)
0	85.42	4.44758
0.5	65.34	4.17960
1	65.40	4.18052
2	48.22	3.87577
3	15.58	2.74599
7	11.88	2.47486
14	4.02	1.39128
30	2.08	0.73237
59	1.03	0.02956

First Half-life (0-3 days) =	1.3 days
Second Half-life (3-59 days) =	14.7 days

SLOPE	-0.072994
RSQ	0.7733747
Intercept	3.617931

First Half-life = 9.5

starting concentration was 1 ppm while max applied concentrations would be need to recalculate 0.05 lb/acre for ppm