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DATA EVALUATION RECORD

STUDY 5

CHEM 057701 Malathion §162-1

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Blumhorst, M. R. 1990. Aerobic soil metabolism study of malathion. Laboratory Report No. 135-004. Unpublished study performed by EPL Bio-Analytical Services, Inc., Harristown, IL, and submitted by the Malathion Reregistration Task Force.

DIRECT REVIEW TIME = 12

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CONCLUSIONS:

Metabolism - Aerobic Soil

1. This study provides supplemental data that shows malathion to be rapidly degraded under aerobic soil metabolism conditions, since the half-life in soil was <1 day. The study is unacceptable at this time for the following reason:

   discrepancies exist between the results of two aerobic soil metabolism experiments. The experiments were apparently identical except that in the "definitive" 92-day experiment the fortification solvent was methanol, and in the supplemental 1-day experiment the fortification solvent was acetone:water. The study authors stated that some degradates may have formed as a result of the fortification solvent.

2. In order for this study to fulfill the aerobic soil metabolism data requirements, the registrant must determine which experiment (methanol or acetone:water fortification solvents) accurately represents the patterns of degradation of malathion and the formation and decline of its degradates. If the use of acetone:water as the fortification solvent is proven to be most accurate, a new study probably will be required, since the experiment was terminated after only 1 day and three sampling intervals.

3. The section below listed as "REVIEWER'S COMMENTS", contain further details of the problems noted with the study.

METHODOLOGY:

Subsamples of Blackoar loam soil (40-46% sand, 40-42% silt,, 14-18% clay, 2.0-2.7% organic matter, pH 6.1, CEC 10.1-10.6 meq/100 g) were sieved (2-mm), weighed into sidearm filter flasks, and adjusted to 75% of field capacity with "Type I" water. The flasks were sealed and incubated in the dark at 22 ± 2° C; during the incubation period, humidified air was drawn through each flask and vented through a polyurethane foam plug and a 1 M sodium hydroxide trapping solution (Figure 1). The air flow through the flasks was maintained at 10 mL/minute. After a minimum of 12 days of incubation, the samples were treated at 6.88 ppm with [2,3-^{14}C]malathion (radiochemical purity 95.6%, specific activity 90 uCi/mg, Amersham) plus unlabeled malathion (purity 98.4%) dissolved in methanol. Soil moisture was adjusted to 75% of field capacity on day 23 posttreatment. Samples were collected immediately posttreatment, at 6 hours, and at 1, 2, 3, 4, 7, 14, and 92 days posttreatment. The sodium hydroxide trapping solutions were replaced at 1, 2, 3, 4, 7, 14, and 92 days posttreatment.

Additional subsamples of the soil were treated at 8.86 ppm with [^{14}C]malathion (radiochemical purity 95.6%, specific activity 90 uCi/mg, Amersham) plus unlabeled malathion (purity 98.4%) dissolved in acetone:water (ratio not reported); samples were collected immediately posttreatment, at 6 hours, and at 1 day posttreatment.

Portions of each soil sample were extracted three times by shaking with acetonitrile:0.05 N hydrochloric acid (90:10, v:v). After each extraction, the slurry was filtered. The extracts were pooled and partitioned twice with methylene chloride, and aliquots of the organic and aqueous fractions were analyzed using LSC. The extracted soil was then Soxhlet-extracted with methanol, and aliquots of the methanol extract were analyzed using LSC. Methylene chloride-, aqueous-, and methanol-extracts containing more than 0.2% of the applied radioactivity were analyzed using one-dimensional TLC on silica gel plates developed twice in benzene:hexane:acetic acid (40:40:10, v:v:v) or hexane:acetic acid:ethyl ether (75:15:10, v:v:v). Unlabeled reference standards were cochromatographed with the samples. Radioactive areas on the plates were visualized using autoradiography; the standards were visualized with iodine. Individual bands were scraped from the plates, [^{14}C]residues were eluted from the silica gel with methanol, and the eluted radioactivity was quantified using LSC. Additional aliquots of the soil extracts were separated by TLC on plates developed as previously described; [^{14}C]residues were scraped from the plates and eluted from the silica gel with acetonitrile:0.05 N hydrochloric acid (90:10, v:v). The solution was filtered and partitioned twice with methylene chloride. The organic phase was concentrated (method not reported) and aliquots were analyzed by HPLC using ODS-1 columns, a mobile phase gradient of acetonitrile:aqueous potassium phosphate buffer, and UV (220 nm) and radioactivity detection. Portions of the extracted soil were air-dried and analyzed for unextracted radioactivity using LSC following combustion.

Subsamples of the Soxhlet-extracted soil were further extracted with sodium hydroxide to determine the relative amounts of fulvic acid-, humic acid-, and humin-associated [^{14}C]residues in the soil. Aliquots of selected extracts were partitioned with methylene chloride, and the organic fraction was concentrated by rotary evaporation and analyzed by HPLC as previously described.

An aliquot of the sodium hydroxide trapping solution was treated with 0.5 M barium chloride and centrifuged. Aliquots of the supernatant were analyzed by LSC. The polyurethane foam plugs were Soxhlet-extracted with methanol; aliquots of the extract were analyzed by LSC.

<u>DATA SUMMARY</u>:

[2,3-^{14}C]Malathion (radiochemical purity 95.6%), at 6.88 and 8.86 ppm, degraded with a registrant-calculated half-life of approximately 0.2 days in two experiments using loam soil that was incubated in the dark at 22 ± 2° C and 75% of field capacity. [^{14}C]Malathion comprised 75.8-97.9% of the applied radioactivity immediately posttreatment, 7.6-36.4% at 6 hours posttreatment, and 2.2-3.4% at 1 day posttreatment (Tables XIII and XXIX). In samples incubated through 92 days posttreatment, [^{14}C]malathion was 0.4-4.0% of the applied radioactivity at 2-14 days and 0.1% at 92 days (Table XIII). The material balances were 85.5-112.5% of the applied through 1 day posttreatment, and 73.7-110.2% with no discernible pattern between 2 and 92 days (Tables XXIII and XXXVI).

Numerous degradates were identified in the soil extracts:

> dicarboxylic acid of malathion was a maximum 18.7-36.7% of the applied;
>
> the alpha monomethyl ester of the dicarboxylic acid of malathion was 3.6-3.8%;
>
> the beta monomethyl ester of the dicarboxylic acid of malathion was 6.0-6.7%;

the dimethyl ester of the dicarboxylic acid of malathion was 4.8-4.9%;

the beta monocarboxylic acid of malathion was 2.8-7.3%;

the monomethyl ester of the beta monocarboxylic acid of malathion was 5.8-6.1%;

the alpha monocarboxylic acid of malathion was 1.9-2.5%; and

malaoxon was 0.6-1.8% (Tables IV-XII, XXVI-XXVIII).

Three degradates were isolated from the soil extracts but were not identified: Unknown 3 at up to 3.7% of the applied radioactivity; Unknown 5 at up to 2.1%; and Unknown 6 at up to 1.0% (Tables IV-XII, XXVI-XXVIII). Extracted [^{14}C]residues that did not migrate from the TLC origin were $\leq$4.2% of the applied radioactivity at all sampling intervals.

In the experiment in which methanol was used as the fortification solvent, unextracted [^{14}C]residues increased from 0.0% of the applied immediately posttreatment to 63.8-64.9% at 1 day, then decreased to 38.5-38.9% by 92 days (Table XXIII). In the experiment in which acetone:water was used as the fortification solvent, unextracted [^{14}C]residues increased from 0.4-0.7% of the applied immediately posttreatment to 21.1-22.9% at 6 hours and 26.2-33.9% at 1 day (Table XXXVI). The further fractionation of the soil organic matter found a maximum of 26.2-31.2%, 9.7-10.5%, and 31.7-34.2% of the applied radioactivity associated with the fulvic acid-, humic acid-, and humin soil-fractions, respectively (Tables XX-XXII and XXXIV).

In the soils incubated for 92 days, total carbon dioxide evolution was 44.8-55.8% of the applied radioactivity; volatile organic residues were $\leq$0.2% (Tables XIV and XVI).

REVIEWER'S COMMENTS:

1. Discrepancies exist between the results of two aerobic soil metabolism experiments, which were apparently identical except that in the "definitive" 92-day experiment the fortification solvent was methanol and in the supplemental 1-day experiment the fortification solvent was acetone:water. The study authors stated that some degradates may have formed as a result of the fortification solvent. For example, Unknowns 5 and 6 were judged to be artifacts of the methanol solvent system used to treat the soil; these compounds were tentatively identified as methyl esters. Also, the concentration of unextracted [^{14}C]residues at 1 day posttreatment was up to 65% of the applied radioactivity in the soil in which the fortification solvent was methanol, compared to 26-34% in the soil fortified using acetone:water.

   The study authors stated that the results of the experiment in which acetone:water was the fortification solvent were most accurate, but did

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not provide any evidence to support this conclusion. Since the acetone:water experiment was of limited duration (1 day) and the soil was only sampled three times, the resulting data are insufficient to satisfy Subdivision N guidelines.

2. One degradate, Unknown 3, was present in the extracts from both the methanol- and acetone:water-treated soils at concentrations up to 3.7% of the applied radioactivity (0.33 ppm). Subdivision N guidelines state that all degradates present at ≥0.01 ppm must be identified.

3. There were several areas of diffuse radioactivity on the TLC plates, comprising 0.04-6.3% of the applied radioactivity.

4. An ancillary experiment was conducted to determine the rate of degradation of malathion in sterile soil using methanol as the carrier. At 4 days posttreatment, malathion comprised >100% of the applied radioactivity (97.84% of the extractable radioactivity). This appears to clearly indicate that microorganisms are responsible for the rapid degradation of malathion in soil under aerobic conditions.

MALATHION

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