5-26-94

MEMORANDUM

SUBJECT: Response to the Malathion Reregistration Standard: Confined Rotational Crop Study. ( Case No. 0248, Chemical I. D. No. 057701,MRID No. 42785501, CBRS No. 12,854, Barcode: D196880).

FROM: R. B. Perfetti, Ph.D., Chemist
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Health Effects Division (7509C)

THRU: W. J. Hazel, Ph.D., Section Head
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Health Effects Division (7509C)

TO: Lois Rossi, Chief
Reregistration Branch
Special Review & Reregistration Division (7508W)

Attached is a review of a confined rotational crop study submitted by A/S Cheminova in response to the malathion Reregistration Standard. This review was completed by Dynamac Corporation under supervision of CBRS, HED. It has undergone secondary review in the branch and has been revised to reflect Agency policies.

1. The submitted confined rotational crop study is adequate. Radioactive residues accumulated ≥0.01 ppm in/on commodities of lettuce, turnip, and wheat that were planted at 30, 120, and 365 days after [2,3-$^{14}$C-succinate]malathion was applied to sandy loam soil at 1.2x the maximum seasonal application rate under field conditions. Accumulation of radioactive residues was lowest in 365-DAT wheat forage (0.01 ppm) and highest in 30-DAT wheat straw (0.42 ppm). For immature lettuce and immature and mature turnips, accumulation of $^{14}$C-residues peaked in commodities from the 120-day rotation. For all other crops, accumulation was highest in commodities from the 30-day rotation and declined thereafter.

2. Much of the residue characterization and identification work was conducted using samples from the 30-day rotation since they contained the highest total radioactive residues. Samples from the 120-day and 365-day rotations were subjected to solvent fractionation schemes; however, characterization and identification of the residues were incomplete. The registrant stated that the majority of the TRR from these rotation intervals remained in polar extracts or were not released during exhaustive extraction.

(1)

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3a. The parent malathion and its metabolite malaoxon are the compounds that need to be regulated in/on plant commodities which are directly treated with malathion according to the HED Metabolism Committee. The metabolite malaoxon was not detected in/on any of the 30-day rotational fractions or extracts. Malathion was identified in the organosoluble fractions of immature lettuce (11.1% TRR, 0.02 ppm), immature turnips (11.9% TRR, 0.03 ppm), and wheat forage (19.9% TRR, 0.02 ppm).

3b. Additional metabolites, malathion monocarboxylic acid (12.0% TRR, 0.03 ppm) and tricarboxylic acid intermediates or sugars (5.3% TRR, <0.04 ppm), were identified in the combined aqueous and potassium phosphate buffer phases of 30-DAT immature turnips. Previously evaluated plant metabolism studies had also identified malathion monocarboxylic acid as a metabolite, but this metabolite is not considered a residue of concern.

3c. Characterized radioactivity in nonextractable fractions of 365-DAT mature turnip roots (84.3% TRR) and 30-DAT wheat grain (78.8% TRR) consisted of sugars, organic acids, starch, protein, pectin, lignin, and hemicellulose and/or cellulose.

4. No further residue characterization and identification are required from the current confined rotational study. However, limited field rotational crop studies (GLN 165-2) are required because malathion was identified in 30-DAT rotational crops and quantified at levels greater than 0.01 ppm. No analyses were conducted on samples of racs at longer plant back intervals. In addition, rotational crop restrictions are needed on malathion end-use product labels. The appropriate plantback intervals will be determined pending submission of the required field rotational crop studies. The required field rotational crop studies should analyze for malathion and its metabolite malaoxon. The Registrant should consult the Pesticide Assessment Guidelines, Subdivision N and the document entitled " Guidance on How to Conduct Studies on Rotational Crops" (EPA 738-B-93-001, 2/93).

A revised Residue Chemistry summary sheet is included.
If you need additional input please advise.

Attachment 1: Malathion Confined Rotational Crop Study Review.

cc (With Attachment 1): RBP, Malathion Reregistration Standard File, Malathion Subject File, Circulation and Dynamac.

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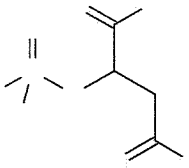
cc (Without Attachment): RF.

MALATHION



Shaughnessy No. 057701; Case 0248

(CBRS No. 12854; DP Barcode D196880)

Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

A/S Cheminova has submitted a confined rotational crop study (1993; MRID 42785501) in support of the reregistration of malathion. This study is evaluated herein for its adequacy in fulfilling reregistration requirements for GLN 165-1 (Confined Rotational Crop Study).

The qualitative nature of malathion residues in plants is adequately understood. The HED Metabolism Committee has concluded that the parent compound malathion and its metabolite malaoxon are the compounds that need to be regulated in/on plant commodities. The qualitative nature of the residue resulting from oral dosing of poultry and ruminants is adequately understood; however, if the direct livestock treatment uses of malathion are supported, then appropriate dermal metabolism and magnitude of the residue in meat, milk, poultry and eggs studies are required.

Tolerances for malathion residues in/on food commodities are currently expressed in terms of malathion *per se* (O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate) [40 CFR §180.111, 40 CFR §185.3850, and 40 CFR §185.7000]. Codex MRLs exist for residues of malathion *per se* in/on various plant and processed commodities. The Codex MRLs and the U.S. tolerances will be incompatible when the U.S. tolerance expression for plant commodities is revised to include both residues of malathion and the metabolite malaoxon.

The Pesticide Analytical Manual (PAM), Vol. II lists a TLC method, a GLC method with potassium chloride thermionic

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detection, and a spectrophotometric method as Methods I, II, and III, respectively, for the enforcement of current malathion tolerances. The Residue Chemistry Science Chapter of the Malathion Reregistration Standard, dated 7/87, recommends use of the GLC method for tolerance enforcement. When the tolerance expression is revised to include both residues of malathion and malaoxon, new enforcement methodology will be required.

CONCLUSIONS AND RECOMMENDATIONS

1. The submitted confined rotational crop study is adequate. Radioactive residues accumulated ≥0.01 ppm in/on commodities of lettuce, turnip, and wheat that were planted at 30, 120, and 365 days after [2,3-$^{14}$C-succinate]malathion was applied to sandy loam soil at 1.2x the maximum seasonal application rate under field conditions. Accumulation of radioactive residues was lowest in 365-DAT wheat forage (0.01 ppm) and highest in 30-DAT wheat straw (0.42 ppm). For immature lettuce and immature and mature turnips, accumulation of $^{14}$C-residues peaked in commodities from the 120-day rotation. For all other crops, accumulation was highest in commodities from the 30-day rotation and declined thereafter.

2. Much of the residue characterization and identification work was conducted using samples from the 30-day rotation since they contained the highest total radioactive residues. Samples from the 120-day and 365-day rotations were subjected to solvent fractionation schemes; however, characterization and identification of the residues were incomplete. The registrant stated that the majority of the TRR from these rotation intervals remained in polar extracts or were not released during exhaustive extraction.

3a. The parent malathion and its metabolite malaoxon are the compounds that need to be regulated in/on plant commodities which are directly treated with malathion according to the HED Metabolism Committee. The metabolite malaoxon was not detected in/on any of the 30-day rotational fractions or extracts. Malathion was identified in the organosoluble fractions of immature lettuce (11.1% TRR, 0.02 ppm), immature turnips (11.9% TRR, 0.03 ppm), and wheat forage (19.9% TRR, 0.02 ppm).

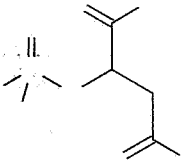
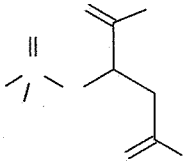
3b. Additional metabolites, malathion monocarboxylic acid (12.0% TRR, 0.03 ppm) and tricarboxylic acid intermediates or sugars (5.3% TRR, <0.04 ppm), were identified in the combined aqueous and potassium phosphate buffer phases of 30-DAT immature turnips. Previously evaluated plant metabolism studies had also identified malathion monocarboxylic acid as a metabolite, but this metabolite is not considered a residue of concern.

3c. Characterized radioactivity in nonextractable fractions of 365-DAT mature turnip roots (84.3% TRR) and 30-DAT wheat grain (78.8% TRR) consisted of sugars, organic acids, starch, protein, pectin, lignin, and hemicellulose and/or cellulose.

4. No further residue characterization and identification are required from the current confined rotational study. However, limited field rotational crop studies (GLN 165-2) are required because malathion was identified in 30-DAT rotational crops and quantified at levels greater than 0.01 ppm. No analyses were conducted on samples of racs at longer plant back intervals. In addition, rotational crop restrictions are needed on malathion end-use product labels. The appropriate plantback intervals will be determined pending submission of the required field rotational crop studies. The required field rotational crop studies should analyze for malathion and its metabolite malaoxon. The Registrant should consult the Pesticide Assessment Guidelines, Subdivision N and the document entitled " Guidance on How to Conduct Studies on Rotational Crops" (EPA 738-B-93-001, 2/93).

The molecular structures of malathion and its metabolite that were characterized and identified in/on rotational crop commodities from this confined rotational crop study are presented in Table 1.

Table 1. Malathion and its metabolites in confined rotational crop commodities (MRID 42785501).

| **Common Name** Chemical Name | Structure | Substrate |
|---|---|---|
| **Malathion** *O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate |  | immature lettuce, immature turnips, wheat forage |
| **Malathion monocarboxylic acid; MCA** *O,O*-dimethyl dithiophosphate of ethyl mercaptosuccinate |  | immature turnips [a] |

[a] Identification not confirmed by a second method.

## DETAILED CONSIDERATIONS

Registered Use Patterns

A REFS search conducted on 1/7/94 identified the following formulations registered to Cheminova on numerous food/feed crops that may be rotated. The 5 lb ai/gal EC formulation (EPA Reg. No. 4787-20, label dated 2/2/89) may be applied using ground (minimum 30 gal/A) or aerial (minimum 5 gal/A) equipment. The 9.79 lb ai/gal ULV formulation (EPA Reg. No. 4787-8, label dated 1/25/91) may be applied undiluted using aerial or ground equipment capable of applying ultra low volume formulations. The 4.1 lb ai/gal RTU formulation (EPA Reg. No. 4787-21, label dated 2/88) may be applied undiluted or diluted with water by ground (minimum 5 gal/A) or aerial equipment (minimum 1 gal/A) capable of applying ultra low volumes. No maximum number of applications per growing season or maximum seasonal rates have been established except as noted in Table 2. No plantback intervals have been established. A summary of malathion use patterns with food/feed ramifications is presented in Table 2.

Table 2. Summary of malathion food/feed use patterns for the 5 lb ai/gal EC, 9.79 lb ai/gal ULV, and 4.1 lb ai/gal RTU formulations (EPA Reg. Nos. 4787-20, 4787-8, and 4787-21, respectively).

| Crops | Single Application Rate (lb ai/A) | Seasonal Rate (lb ai/A) | PHI |
|---|---|---|---|
| Alfalfa and clover | 0.6-1.6 | None specified | 0-5 |
| Cereal grains (barley, rye, oats, and wheat | 0.6-1.3 | None specified | 0-7 |
| Corn | 0.9-1.3 | 6.5 | 5 |
| Cotton | 0.3-2.5 | None specified | 0 |
| Grasses | 0.6-1.3 | None specified | 0 |
| Peanuts | 0.9 | None specified | 0 |
| Rice | 0.6 | None specified | 7 |
| Safflower | 0.6-1.3 | None specified | 3 |
| Sorghum | 0.6-0.9 | None specified | 7 |
| Soybeans | 1.9 | None specified | 0-7 |
| Sugar beets | 0.9-1.9 | None specified | 0-7 |
| Tobacco | 0.9-1.6 | None specified | 0 |
| Vegetables | 0.6-3.4 | None specified | 0-21 |

In-life phase

The in-life phase of the study was conducted at Plant Sciences, Inc. (Watsonville, CA). The test material was prepared by mixing [2,3-$^{14}$C-succinate]malathion (radiochemical purity 97.3%; specific activity 176,975 dpm/µg) with unlabeled malathion in toluene for a final specific activity of 54,969 dpm/µg. A test substance equivalent to a 57% EC formulation was prepared from the radiolabeled material using a blank EC formulation and water. The formulated test substance was applied at a field-equivalent rate of 7.9 lb ai/A (equivalent to 1.2x the maximum seasonal application rate of 6.5 lb ai/A or 2.3x the maximum single

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application rate of 3.4 lb ai/A) to portions of sandy loam soil (63.3% sand, 34.1% silt, 2.6% clay, 2.5% organic matter, pH 6.6, and cation exchange capacity 48 meq/100g) that had been placed in three plastic-lined boxes (2.5 ft wide x 3.0 ft long x 2.0 ft deep). The boxes were filled with soil to within 2 inches of the top. Two additional boxes of soil were used as controls. The test solution was applied using a plastic manual trigger sprayer. After the spray had dried, the test substance was incorporated into the soil to a depth of 10 cm with a small rake. The control boxes of soil were treated with the ready-to-use blank formulation and water. At 30, 120, and 365 days after treatment (DAT; 11/14/90, 2/12/91, and 10/15/91), the pots were planted to lettuce, turnips and wheat. The test boxes were fertilized prior to planting and the crops were watered as necessary; information pertaining to the maintenance schedule (e.g., temperature, humidity, and rainfall) of the crops was provided.

The rotational crops were harvested at the following postplanting intervals: 38-43 days and 63-79 days, respectively, for immature and mature lettuce, 38-50 and 83-166 days, respectively, for immature and mature turnips, 29-64 days for wheat forage, and 141-168 days for wheat straw, grain, and chaff. Lettuce was harvested by cutting the leaves at the soil surface. Turnips were harvested by pulling the entire plant out of the soil and separating the tops from the roots. Mature wheat was harvested and separated into straw, grain, and chaff. The samples were frozen immediately after collection and were shipped overnight to PTRL East, Inc. (Lexington, KY), where they were stored frozen at <5 C prior to processing and analysis. The registrant did not specify the interval between harvest and processing.

Total radioactive residues (TRR)

Subsamples of rotational crops were processed by chopping and grinding with dry ice and were stored frozen until analysis. The TRR in homogenized samples were determined in triplicate by liquid scintillation spectrometry (LSS) following combustion. The limit of quantitation was 0.01 ppm. The TRR in the samples of lettuce, turnips, and wheat forage, straw, grain, and chaff are reported in Table 3.

Table 3. Total radioactive residues (TRR) found in/on rotational crops grown in aged sandy loam soil that had been treated with [2,3-$^{14}$C-succinate]malathion at 1.2x.

| Matrix | TRR (ppm, malathion equivalents) [a] | | |
|---|---|---|---|
| | 30 DAT [b] | 120 DAT | 365 DAT |
| Lettuce (immature) | 0.16 | 0.20 | 0.08 |
| Lettuce (mature) | 0.07 | 0.04 | 0.03 |
| Turnip (immature) | 0.26 | 0.30 | 0.04 |
| Turnip tops (mature) | 0.06 | 0.07 | 0.03 |
| Turnip roots (mature) | 0.05 | 0.11 | 0.06 |
| Wheat forage | 0.14 | 0.09 | 0.01 |
| Wheat straw | 0.42 | 0.28 | 0.05 |
| Wheat grain | 0.27 | 0.15 | 0.05 |
| Wheat chaff | 0.29 | 0.21 | 0.06 |

[a] Average of triplicate analyses.
[b] DAT = Days after treatment.

The TRR data indicate that $^{14}$C-residues accumulated at levels of 0.01 ppm or greater in/on commodities of lettuce, turnips, and wheat that were planted at 30, 120, and 365 days after [2,3-$^{14}$C-succinate]malathion was applied to sandy loam soil at 1.2x under field conditions. Accumulation of radioactive residues was lowest in 365-DAT wheat forage (0.01 ppm) and highest in 30-DAT wheat straw (0.42 ppm). In immature lettuce and immature and mature turnips, accumulation of $^{14}$C-residues peaked in commodities from the 120-day rotation. For all other crops, accumulation was highest in commodities from the 30-day rotation and declined thereafter.

Extraction of $^{14}$C-residues

The registrant provided a flow chart and descriptions of the extraction and fractionation schemes for plant commodities. During these procedures, aliquots of the extracts and solids were analyzed for radioactivity by LSS or combustion/LSS. The extraction and fractionation procedures are described below.

Subsamples of homogenized crop tissues were blended with acidified acetonitrile:water (9.6:1, v:v). The homogenate was filtered and the filtrate was concentrated by rotary evaporation. The nonextractable fraction was blended with methanol:chloroform: acetone (2:1:0.5, v:v:v), and the resulting extract was filtered and concentrated by rotary evaporation. After combining the two organic extracts, 2% sodium sulfate was added and the pH was adjusted to 8.5 with 5% sodium carbonate. The extract was then partitioned three times with chloroform (Chloroform I fraction). The aqueous phase was adjusted to pH 2.0 with 6 N hydrochloric acid and re-partitioned three times with chloroform (Chloroform II fraction). Following

organic extractions, the remaining nonextractable residues were blended with 50 Mm potassium phosphate buffer (pH 7) and filtered. The aqueous and buffer phases from 30-DAT immature turnips were combined, concentrated by lyophilization, and resolubilized in water. The remaining nonextractable residues were dried and reserved for further characterization of bound residues.

Characterization and identification of $^{14}$C-residues

Metabolite profiles in the Chloroform I extracts of 30-DAT immature lettuce, turnips, and wheat forage were examined by HPLC with confirmation by 1-dimensional TLC. The registrant indicated that residues in the organic extracts of the other commodity samples were not analyzed because they did not represent RACs, or the residues would be $<0.01$ ppm if levels were extrapolated to a 1x application rate for malathion *[based on extrapolation to the maximum single application rate of 3.4 lb ai/A]*. The registrant stated further that the majority of the TRR remained in polar extracts or were not released during exhaustive extraction. Prior to HPLC analysis, the Chloroform I and II extracts were concentrated by rotary and nitrogen evaporation, partitioned with acetone, concentrated again, and filtered.

Aliquots of the Chloroform I extracts, and the combined phosphate buffer and aqueous extracts were analyzed separately by HPLC using a Supelco 005 reverse-phase C-18 column (250 mm x 4.6 mm, 5 µm) equipped with a Supelco Pelliguard column (20 mm x 4.6 mm). The mobile phase contained 1% trifluoroacetic acid, and consisted of acetonitrile:water at 0:100 (v:v) changing to 100:0 (v:v) over a period of 100 minutes. Radioactive residues were detected and quantified by LSS analysis of the fractions; unlabeled reference standards were detected by UV at 230 nm. Identification of radiolabeled metabolites was by comparison of relative retention times with those of the following unlabeled reference standards: malathion, malaoxon, malathion dicarboxylic acid, malathion monocarboxylic acid, monoethyl maleate, diethyl maleate, diethyl mercaptosuccinate, diethyl fumarate, diethyl methylthiosuccinate, desmethyl malathion, iso-malathion, malathion mixed ester, and tetraethyl dithiodisuccinate. The HPLC limit of quantitation was $\leq 0.01$ ppm.

Confirmatory analysis for malathion metabolites tentatively identified by HPLC was by 1-dimensional TLC, using silica gel plates and the following solvent systems:

System I: benzene:hexane:acetic acid (2:2:1, v:v:v);
System II: benzene:acetic acid (4:1, v:v);
System III: hexane:acetic acid:diethyl ether (15:3:2, v:v:v); and
System IV: butanol:acetic acid:water (4:1:1, v:v:v).

Samples were co-chromatographed with the reference standards listed above. Radiolabeled metabolites were detected by a TLC plate scanner and were quantified by scraping the the radioactive bands for analysis by LSS. Unlabeled reference standards were visualized under UV light or were derivatized with a 0.5% (w:w) solution of 2,6-dibromoquinone chlorimide in acetone for chromogenic detection.

The aqueous/buffer composite of 30-DAT immature turnips (containing polar $^{14}$C-residues) was cleaned up using semi-preparative HPLC on a Supelcosil column (250 mm x 10 mm, 5

μm) with the same mobile phase and conditions described above. The polar extracts were co-chromatographed with tricarboxylic acid and sugar reference standards on a Biorad Aminex ion exchange column (300 mm x 7.8 mm) equipped with a 30-mm x 4.6-mm guard column. A mobile phase of 0.005 N sulfuric acid was used, and detection was by radioassay or by UV at 210 nm.

Hydrolysis of nonextractable residues

The nonextractable $^{14}C$-residues remaining after organic and phosphate buffer extraction of 365-DAT turnip roots and 30-DAT wheat grain were further analyzed by sequential extraction, fractionation, and hydrolysis to determine the radiocarbon incorporated into endogenous plant constituents. Residues in a subsample of the solid were extracted with 50 Mm potassium phosphate buffer (pH 7) to release radioactivity associated with sugars and organic acids. The remaining residue was incubated with α-amylase for 20 hours at 30 C to release $^{14}C$-residues associated with starch. The remaining solid fraction was incubated in 50 Mm TRIS buffer (pH 7.2) and Pronase E at 30 C for 16 hours to release radioactivity associated with protein. The bound residues that remained following protein digestion were incubated with sodium acetate:EDTA (1:1, w:v; pH 4.5) at 80 C for 6 hours to release $^{14}C$-residues associated with pectin. Following pectin extraction, the remaining residues were incubated with glacial acetic acid:sodium chlorite dihydrate (4:1, v:w) at 70 C for 4 hours to release radioactivity associated with lignin. The solid residues that remained following lignin extraction were incubated with 24% potassium hydroxide at 27 C for 24 hours, and then neutralized with 6 N acetic acid to release radioactivity present as hemicellulose. The remaining residue was then hydrolyzed with 72% sulfuric acid at ambient temperature for 4 hours to release $^{14}C$-residues present as cellulose. The remaining solid residues and the filters used in the filtration steps were analyzed by combustion/LSS to determine the total nonextractable radioactivity. None of these cell fractionation procedures was supported by a confirmatory technique.

The quantitative results of the characterization/identification procedures in rotational crop commodities are presented in Table 4 (lettuce), Table 5 (turnips) and Table 6 (wheat). Summaries of the results of characterization/identification are presented in Table 7 (lettuce and turnip) and Table 8 (wheat).

Table 4. Distribution of total radioactive residues (TRR) in/on **lettuce** grown in aged sandy loam soil treated with [2,3-$^{14}$C-succinate]malathion at 1.2x.

| Fraction [a] | % TRR | ppm | Characterization/Identification [b] |
|---|---|---|---|
| **30-DAT immature lettuce (0.16 ppm)** | | | |
| Chloroform I | 18.1 | 0.03 | Malathion (11.1% TRR, 0.02 ppm) was identified. |
| Chloroform II | 5.5 | <0.01 | N/A = Not analyzed |
| Aqueous | 16.4 | 0.03 | N/A |
| Phosphate buffer | 10.4 | 0.02 | N/A |
| Nonextractable | 42.8 | 0.07 | N/A |
| **30-DAT mature lettuce (0.07 ppm)** | | | |
| Chloroform I | 10.4 | <0.01 | N/A |
| Aqueous | 40.8 | 0.03 | N/A |
| Phosphate buffer | 4.3 | <0.01 | N/A |
| Nonextractable | 50.5 | 0.04 | N/A |
| **120-DAT mature lettuce (0.04 ppm)** | | | |
| Chloroform I | 24.8 | 0.01 | N/A |
| Aqueous | 42.6 | 0.02 | N/A |
| Phosphate buffer | 17.5 | 0.01 | N/A |
| Nonextractable | 104.3 | 0.04 | N/A |
| **365-DAT mature lettuce (0.03 ppm)** | | | |
| Chloroform I | 26.3 | 0.01 | N/A |
| Chloroform II | 16.1 | <0.01 | N/A |
| Aqueous | 17.6 | 0.01 | N/A |
| Phosphate buffer | 18.0 | 0.01 | N/A |
| Nonextractable | 71.6 | 0.02 | N/A |

[a] Only those fractions containing detectable radioactivity are reported herein.

[b] Metabolites were identified by HPLC and confirmed by 1-dimensional TLC.

Table 5. Distribution of total radioactive residues (TRR) in/on **turnips** grown in aged sandy loam soil treated with [2,3-$^{14}$C-succinate]malathion at 1.2x.

| Fraction [a] | % TRR | ppm | Characterization/Identification [b] |
|---|---|---|---|
| **30-DAT immature turnips (0.26 ppm)** | | | |
| Chloroform I | 15.9 | 0.04 | Malathion (11.9% TRR, 0.03 ppm) was identified. |
| Chloroform II | 5.9 | 0.02 | N/A = Not analyzed |
| Aqueous | 13.7 | 0.04 | Malathion monocarboxylic acid (12.0% TRR, 0.03 ppm) was identified by HPLC from the composite aqueous and buffer extract. Citrate (2.4% TRR, <0.01 ppm), succinate (0.7% TRR, <0.01 ppm), fumarate (1.0% TRR, <0.01 ppm), and other compounds (1.2% TRR, <0.01 ppm; sucrose, glucose, and/or malic acid) were identified by ion-exchange chromatography. [c] |
| Phosphate buffer | 20.9 | 0.05 | |
| Nonextractable | 56.9 | 0.15 | N/A |
| **30-DAT mature turnip tops (0.06 ppm)** | | | |
| Chloroform I | 19.3 | 0.01 | N/A |
| Aqueous | 18.9 | 0.01 | N/A |
| Phosphate buffer | 11.9 | 0.01 | N/A |
| Nonextractable | 32.8 | 0.02 | N/A |
| **120-DAT mature turnip tops (0.07 ppm)** | | | |
| Chloroform I | 13.6 | 0.01 | N/A |
| Aqueous | 13.1 | 0.01 | N/A |
| Phosphate buffer | 15.3 | 0.01 | N/A |
| Nonextractable | 48.9 | 0.03 | N/A |
| **365-DAT mature turnip tops (0.03 ppm)** | | | |
| Chloroform I | 22.2 | 0.01 | N/A |
| Aqueous | 12.0 | <0.01 | N/A |
| Phosphate buffer | 14.4 | <0.01 | N/A |
| Nonextractable | 49.6 | 0.02 | N/A |
| **30-DAT mature turnip roots (0.05 ppm)** | | | |
| Aqueous | 31.6 | 0.02 | N/A |
| Nonextractable | 39.4 | 0.02 | N/A |
| **120-DAT mature turnip roots (0.11 ppm)** | | | |
| Chloroform I | 6.3 | 0.01 | N/A |
| Aqueous | 10.6 | 0.01 | N/A |
| Phosphate buffer | 10.1 | 0.01 | N/A |
| Nonextractable | 49.3 | 0.05 | N/A |

*(continued; footnotes follow)*

Table 5 *(continued)*.

| | | | |
|---|---|---|---|
| **365-DAT mature turnip roots (0.06 ppm)** | | | |
| Aqueous | 7.8 | <0.01 | N/A |
| Phosphate buffer | 9.1 | 0.01 | N/A |
| Nonextractable | 66.3 | 0.04 | Conducted cell wall fractionation. |
| Phosphate buffer wash | 6.6 | <0.01 | Characterized as sugars and organic acids. |
| Amylase hydrolysate | 9.9 | 0.01 | Characterized as starch. |
| Pronase E hydrolysate | 17.2 | 0.01 | Characterized as protein. |
| EDTA hydrolysate | 9.8 | <0.01 | Characterized as pectin. |
| Acetic acid/sodium chlorite | 8.9 | <0.01 | Characterized as lignin. |
| Potassium hydroxide hydrolysate | 9.0 | 0.01 | Characterized as hemicellulose. |
| Sulfuric acid hydrolysate | 22.9 | 0.01 | Characterized as cellulose. |
| Nonextractable | 5.0 | <0.01 | N/A |

[a] Only those fractions containing detectable radioactivity are reported herein.
[b] Metabolites were identified by HPLC and confirmed by 1-dimensional TLC.
[c] %TRR and ppm values were calculated by the study reviewer.

*(continued; footnotes follow)*

Table 6. Distribution of total radioactive residues (TRR) in/on **wheat** grown in aged sandy loam soil treated with [2,3-$^{14}$C-succinate]malathion at 1.2x.

| Fraction [a] | % TRR | ppm | Characterization/Identification [b] |
|---|---|---|---|
| **30-DAT wheat forage (0.14 ppm)** | | | |
| Chloroform I | 33.4 | 0.05 | Malathion (19.9% TRR, 0.02 ppm) was identified. |
| Aqueous | 51.1 | 0.07 | N/A = Not analyzed |
| Phosphate buffer | 13.2 | 0.02 | N/A |
| Nonextractable | 63.4 | 0.09 | N/A |
| **120-DAT wheat forage (0.09 ppm)** | | | |
| Chloroform I | 32.2 | 0.03 | N/A |
| Aqueous | 19.1 | 0.02 | N/A |
| Phosphate buffer | 11.3 | 0.01 | N/A |
| Nonextractable | 46.8 | 0.04 | N/A |
| **365-DAT wheat forage (0.01 ppm)** | | | |
| Chloroform I | 67.9 | 0.01 | N/A |
| Phosphate buffer | 23.6 | 0.02 | N/A |
| Nonextractable | 64.6 | 0.01 | N/A |
| **30-DAT wheat straw (0.42 ppm)** | | | |
| Chloroform I | 4.1 | 0.02 | N/A |
| Chloroform II | 1.1 | <0.01 | N/A |
| Aqueous | 8.1 | 0.03 | N/A |
| Phosphate buffer | 2.9 | 0.01 | N/A |
| Nonextractable | 71.8 | 0.30 | N/A |
| **120-DAT wheat straw (0.28 ppm)** | | | |
| Chloroform I | 4.9 | 0.01 | N/A |
| Aqueous | 7.0 | 0.02 | N/A |
| Phosphate buffer | 1.8 | 0.01 | N/A |
| Nonextractable | 95.9 | 0.27 | N/A |
| **365-DAT wheat straw (0.05 ppm)** | | | |
| Chloroform I | 10.7 | 0.01 | N/A |
| Aqueous | 19.0 | 0.01 | N/A |
| Phosphate buffer | 5.7 | <0.01 | N/A |
| Nonextractable | 149.2 | 0.07 | N/A |
| **30-DAT wheat grain (0.27 ppm)** | | | |
| Chloroform I | 5.1 | 0.01 | N/A |
| Aqueous | 1.5 | <0.01 | N/A |
| Phosphate buffer | 5.4 | 0.01 | N/A |
| Nonextractable | 78.8 | 0.21 | Conducted cell fractionation. |
| Phosphate buffer wash | <0.10 | <0.01 | N/A |
| Amylase hydrolysate | 30.3 | 0.08 | Characterized as starch. |
| Pronase E hydrolysate | 27.0 | 0.07 | Characterized as protein. |
| EDTA hydrolysate | <0.10 | <0.01 | N/A |
| Acetic acid/sodium chlorite hydrolysate | <0.10 | <0.01 | N/A |

*(continued; footnotes follow)*

Table 6 *(continued)*.

| | | | |
|---|---|---|---|
| Potassium hydroxide hydrolysate | 11.7 | 0.03 | Characterized as hemicellulose. |
| Sulfuric acid hydrolysate | 37.0 | 0.10 | Characterized as cellulose. |
| Nonextractable | <0.10 | <0.01 | N/A |
| **120-DAT wheat grain (0.15 ppm)** | | | |
| Chloroform I | 4.5 | 0.01 | N/A |
| Chloroform II | 1.6 | <0.01 | N/A |
| Phosphate buffer | 2.9 | <0.01 | N/A |
| Nonextractable | 75.8 | 0.11 | N/A |
| **365-DAT wheat grain (0.05 ppm)** | | | |
| Phosphate buffer | 5.0 | <0.01 | N/A |
| Nonextractable | 83.5 | 0.04 | N/A |
| **30-DAT wheat chaff (0.29 ppm)** | | | |
| Chloroform I | 4.4 | 0.01 | N/A |
| Aqueous | 5.4 | 0.02 | N/A |
| Phosphate buffer | 9.1 | 0.03 | N/A |
| Nonextractable | 98.5 | 0.27 | N/A |
| **120-DAT wheat chaff (0.21 ppm)** | | | |
| Chloroform I | 5.3 | 0.01 | N/A |
| Aqueous | 3.3 | 0.01 | N/A |
| Phosphate buffer | 3.5 | 0.01 | N/A |
| Nonextractable | 80.4 | 0.17 | N/A |
| **365-DAT wheat chaff (0.06 ppm)** | | | |
| Aqueous | 5.7 | <0.01 | N/A |
| Phosphate buffer | 14.6 | 0.01 | N/A |
| Nonextractable | 68.4 | 0.04 | N/A |

[a] Only those fractions containing detectable radioactivity are reported herein.

[b] Metabolites were identified by HPLC and confirmed by 1-dimensional TLC.

*(continued; footnotes follow)*

Table 6 *(continued)*.

Table 7. Summary of characterization/identification of radioactive residues (TRR) in/on **lettuce** and **turnips** grown in aged sandy loam soil treated with [2,3-$^{14}$C-succinate]malathion at 1.2x.

| ion [a] | Immature Lettuce | | Lettuce | | Immature Turnips | | Turnip Tops | | Turnip R |
|---|---|---|---|---|---|---|---|---|---|
| tabolite | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR |
| **30 DAT** | | | | | | | | | |
| osoluble | 23.6 | <0.04 | 10.4 | <0.01 | 21.8 | 0.06 | 19.3 | 0.01 | -- |
| lathion | 11.1 | 0.02 | -- | -- | 11.9 | 0.03 | -- | -- | -- |
| us soluble | 26.8 | 0.05 | 45.1 | <0.04 | 34.6 | 0.09 | 30.8 | 0.02 | 31.6 |
| lathion nocarboxylic | -- | -- | - | - | 12.0 | 0.03 | -- | -- | -- |
| icarboxylic acid termediates | -- | -- | -- | -- | 5.3 | <0.04 | -- | -- | -- |
| tractable | 42.8 | 0.07 | 50.5 | 0.04 | 56.9 | 0.15 | 32.8 | 0.02 | 39.4 |
| **120 DAT** | | | | | | | | | |
| osoluble | -- | -- | 24.8 | 0.01 | -- | -- | 13.6 | 0.01 | 6.3 |
| us soluble | -- | -- | 60.1 | 0.03 | -- | -- | 28.4 | 0.02 | 20.7 |
| tractable | -- | -- | 104.3 | 0.04 | -- | -- | 48.9 | 0.03 | 49.3 |
| **365 DAT** | | | | | | | | | |
| osoluble | -- | -- | 42.4 | <0.02 | -- | -- | 22.2 | 0.01 | 7.8 |
| us soluble | -- | -- | 35.6 | 0.02 | -- | -- | 26.4 | <0.02 | 9.1 |
| tractable | -- | -- | 71.6 | 0.02 | -- | -- | 49.6 | 0.02 | 66.3 |
| gars + organic | -- | -- | -- | -- | -- | -- | -- | -- | 6.6 |
| arch | -- | -- | -- | -- | -- | -- | -- | -- | 9.9 |
| otein | -- | -- | -- | -- | -- | -- | -- | -- | 17.2 |
| ctin | -- | -- | -- | -- | -- | -- | -- | -- | 9.8 |
| gnin | -- | -- | -- | -- | -- | -- | -- | -- | 8.9 |
| micellulose | -- | -- | -- | -- | -- | -- | -- | -- | 9.0 |
| llulose | -- | -- | -- | -- | -- | -- | -- | -- | 22.9 |
| nextractable | -- | -- | -- | -- | -- | -- | -- | -- | 5.0 |

[a] Organosoluble = Chloroform I + II extracts; aqueous soluble = aqueous + buffer extracts.

*(continued; footnotes follow)*

Table 6 *(continued)*.

Table 8. Summary of characterization/identification of radioactive residues (TRR) in/on **wheat** grown in aged sandy loam soil treated with [2,3-$^{14}$C-succinate]malathion at 1.2x.

| on and metabolite [a] | Wheat Forage | | Wheat Straw | | Wheat Grain | | Wheat Cha | |
|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | |
| **30 DAT** | | | | | | | | |
| soluble | 33.4 | 0.05 | 5.2 | <0.03 | 5.1 | 0.01 | 4.4 | 0 |
| athion | **19.9** | **0.02** | -- | -- | -- | -- | -- | |
| s soluble | 64.3 | 0.09 | 11.0 | 0.04 | 6.9 | <0.2 | 14.5 | 0 |
| ractable | 63.4 | 0.09 | 71.8 | 0.30 | 78.8 | 0.21 | 98.5 | 0 |
| rch | -- | -- | -- | -- | 30.3 | 0.08 | -- | |
| tein | -- | -- | -- | -- | 27.0 | 0.07 | -- | |
| icellulose | -- | -- | -- | -- | 11.7 | 0.03 | -- | |
| lulose | -- | -- | -- | -- | 37.0 | 0.10 | -- | |
| extractable | -- | -- | -- | -- | <0.10 | <0.01 | -- | |
| **120 DAT** | | | | | | | | |
| soluble | 32.2 | 0.03 | 4.9 | 0.01 | 6.1 | <0.02 | 5.3 | 0 |
| s soluble | 30.4 | 0.03 | 8.8 | 0.03 | 2.9 | <0.01 | 6.8 | 0 |
| ractable | 46.8 | 0.04 | 95.9 | 0.27 | 75.8 | 0.11 | 80.4 | 0 |
| **365 DAT** | | | | | | | | |
| soluble | 67.9 | 0.01 | 10.7 | 0.01 | -- | -- | 5.7 | < |
| s soluble | 23.6 | 0.02 | 24.7 | <0.02 | 5.0 | <0.01 | 14.6 | 0 |
| ractable | 64.6 | 0.01 | 149.2 | 0.07 | 83.5 | 0.04 | 68.4 | 0 |

[a] Organosoluble = Chloroform I + II extracts; aqueous soluble = aqueous + buffer extracts.

*(continued; footnotes follow)*

Table 6 *(continued)*.

*(continued; footnotes follow)*

Table 6 *(continued)*.

Storage stability

Dates of TRR analyses, extraction, and characterization of residues were not provided by the registrant for any of the rotational crop commodities except 30-DAT immature turnips. For this commodity, the registrant provided data from a concurrent storage stability study. Sample HPLC chromatograms were provided. Subsamples of 30-DAT immature turnips, stored frozen at an unspecified temperature, were extracted and analyzed by HPLC 250 days (8 months) after harvest and 746 days (25 months) after harvest using the same methods described for samples in the metabolism study. The data indicate that the residue profile in immature turnips did not change over 17 months of frozen storage. The Chloroform I extracts contained similar levels of radioactivity and malathion residues.

Study summary

In summary, the present confined rotational crop study is adequate. Radioactive residues accumulated ≥0.01 ppm in/on commodities of lettuce, turnips, and wheat that were planted at 30, 120, and 365 days after [2,3-$^{14}$C-succinate]malathion was applied to sandy loam soil at 1.2x the maximum seasonal application rate under field conditions.

Much of the residue characterization and identification work was conducted using samples from the 30-day rotation since these samples contained the highest level of total radioactive residues. The metabolite malaoxon was not detected in/on any of the 30-day rotational fractions or extracts. Malathion was identified in the organosoluble fractions of immature lettuce (11.1% TRR, 0.02 ppm), immature turnips (11.9% TRR, 0.03 ppm), and wheat forage (19.9% TRR, 0.02 ppm). Additional metabolites, malathion monocarboxylic acid (12.0% TRR, 0.03 ppm) and tricarboxylic acid intermediates or sugars (5.3% TRR, <0.04 ppm), were identified in the combined aqueous and phosphate buffer phases of 30-DAT immature turnips. *[Note: Previous plant metabolism studies also identified malathion monocarboxylic acid in plant matrices, but the metabolite is not considered a residue of concern.]* Characterized radioactivity in nonextractable fractions of 365-DAT mature turnip root (84.3% TRR) and 30-DAT wheat grain (78.8% TRR) was associated with sugars, organic acids, starch, protein, pectin, lignin, and hemicellulose and/or cellulose.

No further residue characterization and identification are required from the current confined rotational study. However, limited field rotational crop studies (GLN 165-2) are required because malathion was identified in 30-DAT rotational crops and quantified at levels greater than 0.01 ppm. No analyses were conducted on samples of racs at longer plant back intervals. In addition, rotational crop restrictions are needed on malathion end-use product labels. The appropriate plantback intervals will be determined pending submission of the required field rotational crop studies.

*(continued; footnotes follow)*

Table 6 *(continued)*.

MASTER RECORD IDENTIFICATION NUMBERS

The citation for the MRID document referred to in this review is presented below.

425785501 Wootton, M.; Johnson, T. (1993) A Confined Rotational Crop Study with $^{14}$C-Malathion Using Turnips (*Brassica rapa*), Lettuce (*Lactusa sativa*), and Wheat (*Triticum aestivum*). Laboratory Project ID: PTRL Project No. 422; PTRL Report No. 1506. Unpublished study conducted by Pharmacology Toxicology Research Laboratory, Lexington, KY and submitted by Cheminova Agro A/S. 176 p.

*(continued; footnotes follow)*