UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 28 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Monosodium methanearsonate (MSMA). List B Reregistration Case No. 2395. Metabolism Studies in Cotton and Citrus (Lemon). MRID Nos. 422161-00, 422161-01, 423244-00, 423244-01, 423912-00, and 423912-01. CBRS Nos. 9525, 9942, and 10245. DP Barcode Nos. D175070, D178793, and D180717.

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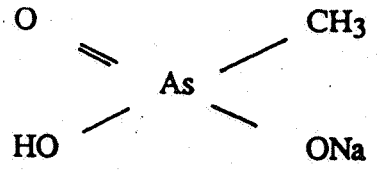
On behalf of the MAA (MSMA/DMSA) Research Task Force Three (EPA Reg. No. 64109), Chemical Consultants International, Inc. (CCII) has submitted ^{14}C-monosodium methanearsonate metabolism studies in cotton (MRID Nos. 422161-00 and -01) and citrus (lemon) [MRID Nos. 423244-00 and -01]. The performing laboratory for all analytical work was PTRL East, Inc. of Richmond Kentucky. The in-life phase of the cotton study was conducted by PTRL in Kentucky, while the in-life phase of the citrus study was conducted in Richmond, California.

Tolerances are currently established for the selective post-emergence herbicide methanearsonic acid (calculated as As_2O_3) resulting from application of the disodium and monosodium salts of methanearsonic acid in or on cottonseed (0.7 ppm) and in or on citrus fruit (0.35 ppm) [40 CFR §180.289]. A tolerance of 0.9 ppm (expressed as As_2O_3) is established for residues of methanearsonic acid in cottonseed hulls from application of the disodium and monosodium salts of methanearsonic acid in the production of cotton [40 CFR §186.4050].

The methanearsonic acid salts comprise List B reregistration case no. 2395. A Phase 4 review was completed 3/28/91 (memo, C. Olinger, CBRS Nos. 6974, 7058, 7097, and 7215), in which plant metabolism studies were required for cotton, a grass, and a citrus fruit. The structure of MSMA is:



MSMA

Conclusions: Cotton Metabolism

1. Cotton plants were treated with monosodium methanearsonate (MSMA) labelled with ^{14}C in the methyl group. Adequate supporting documentation regarding the radiochemical purity of the test substance was submitted.

2. The preparation of treatment solutions applied to cotton was adequately described. The treatment solutions applied on separate dates contained 381 and 380.4 mg ^{14}C[MSMA] diluted in water, with a small quantity of Triton X-100 surfactant. The rates used correspond to 1.1X treatments, made at mid-bloom, rather than prior to first bloom as specified on registered labels. The slightly exaggerated rate was used to ensure that adequate residues would be obtained.

3. The description of the greenhouse conditions under which cotton was grown, including temperature, irrigation, and pest control was adequate.

4. Sample harvest was adequately described: bolls and leaves were cut from the plant stems, and the samples placed in frozen storage. Sample preparation involved manual separation of the cottonseed from the bolls and lint; cottonseed and lint were ground with dry ice prior to analysis.

5. The total radioactive residue (TRR) in seeds and leaves was determined using combustion, followed by liquid scintillation counting (LSC). Radioactive residues in cottonseed alone were characterized; this is acceptable since seed contained 1.49 ppm of radioactivity, while leaves contained only 0.88 ppm radioactivity. Furthermore, registered labels contain restrictions against the feeding of cotton foliage to livestock.

6. Cottonseed was subjected to solvent extraction, followed by acid and base hydrolysis. Metabolites were characterized and/or identified using HPLC, TLC, and GC/MS.

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Instrument conditions were adequately documented.

7. Cottonseed aqueous extracts contained 56% of the cottonseed TRR. The hexane extract (fraction containing cottonseed oil) contained 5% of the seed TRR. Aqueous fractions of acid and base hydrolysates contained 9% and 5% of the TRR, respectively. Organic fractions of acid and base hydrolysates, as well as the methanol extract, each contained less than 2% of the seed TRR. Bound residues constituted 9% of the seed TRR. A total of 88% of the seed radioactivity was recovered through the extraction and hydrolysis procedures.

8. Residues of [^{14}C]MSMA accounted for 27% of the seed TRR, while residues of [^{14}C]cacodylic acid accounted for only 6% of the seed TRR.

9a. Residues of two unidentified metabolites, Unknown 1 and Unknown 2 accounted for 10% and 24% of the seed TRR. Attempts at characterization of Unknown 1 were unsuccessful; however, the study report claimed that Unknown 1 contained [^{14}C]MSMA.

9b. Further characterization of Unknown 2 was attempted using a soil metabolite exhibiting HPLC characteristics similar to those of Unknown 2 found in the cottonseed aqueous extract. The registrant concluded that Unknown 2 contained MSMA, based on HPLC and GC/MS data. While the GC/MS data confirm the presence of MSMA, additional data pertaining to the soil extraction procedure and HPLC analyses must be submitted. Specifically, the registrant must submit the details of the soil extract analytical work, including a discussion of why the retention time for Unknown 2 changed so dramatically within the same chromatographic system when chromatographed a second time.

10. Adequate supporting documentation/chromatograms were submitted for all analytical work (other than the soil extract; see Conclusion 9b).

11. Pending receipt of the required soil extraction and analysis data, CBRS tentatively concludes that the residues of concern in cottonseed appear to include MSMA and cacodylic acid, MSMA being the primary residue of concern.

Conclusions: Citrus (Lemon) Metabolism

1. Lemons trees were treated with monosodium methanearsonate (MSMA) labelled with ^{14}C in the methyl group. Adequate supporting documentation regarding the radiochemical purity of the test substance was submitted.

2. The preparation of treatment solutions applied to lemon trees was adequately described. The treatment solutions applied on 3 separate dates contained 54-58 mg ^{14}C[MSMA], for a total of approximately 175 mg/tree during the course of the study. The rate used in the 3 applications corresponds to 4.86 lb ai/A, the maximum rate currently allowed on the registered label for disodium methanearsonate (DMSA) Drexel 81P [EPA Reg. No.

19713-113].

3. Sample harvest included picking the leaves and lemons on the trees; 90% of the lemons were mature at harvest, while 10% were light green to yellow in color. Samples were frozen following harvest.

4. Sample preparation was adequately described. Lemons were manually separated into peel, pulp, seed, and juice samples.

5. The total radioactive residue (TRR) in each sample was determined by combustion to $^{14}CO_2$ followed by liquid scintillation counting (LSC). Lemon peel contained 0.44 ppm of radioactivity (53% of the fruit TRR); lemon pulp contained 0.07 ppm of radioactivity (24% of the TRR); and lemon juice contained 0.12 ppm (23% of the fruit TRR).

6. Lemon matrices were subjected to solvent extraction. Neither acid nor base hydrolysis was required to release additional radioactive residues.

7. In the lemon peel, 94% of the radioactivity was identified as MSMA (40.5%, including Unknown 1) and cacodylic acid (54.2%). Unidentified radioactivity consisted of Unknown 3 (0.5%). In lemon pulp, 97% of the radioactivity was identified as MSMA (35.8%, including Unknown 1) and cacodylic acid (61.2%). In lemon juice, 92.1% of the radioactivity was identified as MSMA (40.3%, including Unknown 1) and cacodylic acid (51.8%); Unknown 2 comprised 7.9% of the juice radioactivity.

8. Adequate supporting documentation/chromatograms were included in the lemon metabolism study report.

9. CBRS concludes that the residues of concern in lemons include MSMA and cacodylic acid. While cacodylic acid comprised a greater percentage of the TRR in all matrices, both MSMA and cacodylic acid residues can be considered equally significant.

Recommendation

The registrant should submit HPLC radiochromatograms from soil extracts, along with any other information needed to confirm that the unknown metabolites identified in the soil correspond to Unknowns 1 and 2 found in the cottonseed aqueous extract. In addition, the registrant should submit a detailed discussion of the possible reasons for the significant change in the retention time (34 minutes to 12 minutes) of Unknown 2, isolated from the soil extract, observed on the HPLC radiochromatogram included in the report.

Cottonseed and lemon (peel, pulp, juice) generated in the metabolism studies must be analyzed using the data collection and enforcement methods, in order to verify the metabolite identifications, and to ensure that these methods detect all residues of concern. The registrant must analyze the cottonseed for total As, to confirm that all of the seed ^{14}C exists associated with

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arsenic. Such experiments, combined with the results of radiovalidation studies, could eliminate the need for additional analytical work/discussion relating to the soil metabolites and poor characterization of residues.

DETAILED CONSIDERATIONS

Cotton Metabolism Study: MRID No. 42216101

Radiolabelled Test Substance and Application to Cotton Plants

Monosodium methanearsonate (MSMA) was labelled with ^{14}C in the methyl group; the radiolabelled material was obtained from Ricera, Inc. The specific activity of the [^{14}C]MSMA was 2.4 mCi/mmole, with a radiochemical purity of 100%, as determined by HPLC. Adequate supporting data were submitted. The specific activity of the treatment solution applied to cotton plants was 2.3 mCi/mmole.

Cotton plants grown in pots at PTRL's greenhouse facility in Richmond Kentucky were treated twice mid-bloom. The physical characteristics of the silt loam soil were adequately described. Although the protocol indicated that the soil would be analyzed for non-labelled arsenic, the method proposed/used was not reproducible, and therefore was not further utilized. The cotton test crop, *Gossypium hirsutum*, was seeded on 7/5/90, and harvested on 12/26/90. The two mid-bloom treatments were applied to the soil as a spray using a manual pipette, in order to simulate the directed spray application specified on the registered label for Target MSMA 6.6. The treatments were made on 9/7/90 and 9/20/90, corresponding to 110 and 97 days PHI, respectively. Cotton plants were harvested at maturity, on 12/26/90. The material applied to the cotton consisted of the [^{14}C]MSMA diluted in water, with a small amount of Triton X-100. The solution applied to control plants consisted of water with Triton X-100.

The application rate was chosen to approximate the maximum rate according to registered labels. Calculations were based on cotton plant density in commercial fields. An additional 10% MSMA was added to the rate, in order to insure maximum registered label rates were achieved. The rate used in the study was 2.27 lb a.i./A, while current labeling specifies 2.06 lb a.i./A. Consequently, cotton plants were treated twice at a rate of approximately 1.1X. Registered labels stipulate that the 2 treatments should be made prior to first bloom, while the treatments in the study were made mid-bloom. A total of 381 mg [^{14}C]MSMA were applied during treatment 1, while 380.4 mg were applied in treatment 2. A total of 12 cotton plants/pot were included in the radiolabel treatment group, while 6 plants/pot were included in the control group.

The study report included adequate reporting of temperature, humidity, and watering schedules for the duration of the study. Sample harvest entailed cutting the bolls and leaves from the plant stems. Samples were placed in separate bags, and placed into frozen storage. Cottonseeds were separated from the bolls and lint manually (although the protocol had specified

mechanical ginning as the method to be used); seeds and leaves were ground with dry ice, weighed, and placed in frozen storage. Daily records for freezers in which the radioactive samples were stored were not submitted. The study protocol indicated that samples would be processed and analyzed within 30 days of harvest; however, in a deviation from the protocol, it was noted that samples had been processed within 30 days, but were not analyzed until 69 days following harvest.

The Total Radioactive Residue (TRR) in seeds and leaves was determined using combustion, followed by Liquid Scintillation Counting (LSC). The radioactivity detected in cottonseed was 1.49 ppm, while leaves contained 0.88 ppm of radioactivity (the report did not state whether radioactivity was determined in stems, bolls, and linters). Only the residues in cottonseed were fully characterized. Since current labelling prohibits the feeding of treated cotton foliage to livestock, the registrant did not attempt to characterize these residues.

Extraction and Hydrolysis Procedure

Cottonseed samples were extracted first by stirring with water; 5-gram samples were stirred for 2 hours, filtered, and the process repeated 2 times. Water filtrates were combined and set aside for analysis. The remaining solids were extracted in a similar manner, first with methanol (MeOH), and then with hexane. In each case, solids were extracted 3 times, and the filtrates combined for analysis. The hexane fraction was evaporated to remove the solvent, leaving only cottonseed oil. The remaining solids were subjected to an acid hydrolysis step, which involved refluxing in 0.1N HCl for 6 hours. The hydrolysate was partitioned with dichloromethane (DCM), and the organic and aqueous fractions set aside. The solids were then subjected to a base hydrolysis step, during which the solids were refluxed with 0.1N ammonium hydroxide (NH_4OH) for 5 hours. The base hydrolysate was partitioned with DCM, and the aqueous and organic fractions set aside for analysis. The remaining filter cake was oxidized for quantitation of bound residues using LSC. A total of 88% of the cottonseed radioactivity was recovered through the extraction procedure. Table 1 provides a summary of the cottonseed %TRR recovered from each of the fractions generated during the extraction procedure.

Table 1. Distribution of Radioactive Residues in Cottonseed Extracts*

	Solvent Extract			Acid Hydrolysis		Base Hydrolysis			Method
Sample Description	H_2O % ppm	MeOH % ppm	Hexane % ppm	Organic % ppm	Aqueous % ppm	Organic % ppm	Aqueous % ppm	Bound % ppm	Recovery % ppm
Cottonseed	56 0.83	2 0.03	5 0.07	1 0.01	9 0.13	1 0.01	5 0.07	9 0.13	88 1.31

* Residues are expressed as % of the cottonseed TRR; ppm radioactivity are also reported. The cottonseed TRR was 1.49 ppm.

Analytical Techniques used for Characterization and Identification of Radioactive Residues

Cottonseed extracts were analyzed using HPLC, in order to identify or characterize radioactive residues. The identities of radiolabelled components separated by HPLC were confirmed using TLC. Confirmation of the identification of MSMA and CA (cacodylic acid) in cottonseed was accomplished by derivatization, followed by GC/MS analysis. Each of these systems is described briefly:

HPLC: The retention time of unlabelled MSMA, as well as the radiochemical purity of the test [^{14}C]MSMA, was determined using an HPLC system consisting of a Spectra Physics LC pump, an LC-18 reverse-phase column, a Micromeritics variable UV detector, and a Radiomatic radioactivity flow monitor. Cottonseed extracts were analyzed using a system comprised of a Spectra Physics LC pump, an LC-18 reverse-phase column, a Micromeritics variable UV detector, and an automated fraction collector. Radiochromatograms were generated by computer following LSC analysis of the collected fractions. The limit of quantitation (LOQ) for radioactive residues in cottonseed ranged from 0.01 to 0.04 ppm.

TLC: Cottonseed extracts were analyzed on cellulose plates, in a solvent system consisting of ethyl acetate/acetic acid/water (3/2/1). Following development of the TLC plates, radiolabelled compounds were detected using X-ray film, or using a Bioscan Imaging scanner. Selected plates were scraped, and analyzed using oxidation followed by LSC.

GC/MS: Prior to derivatization with thioglycolic acid methyl ester to form the thiomethylglycolate derivative of both MSMA and CA, cottonseed extracts were adjusted to pH 3.5 with a citrate/phosphate buffer. Derivatives were partitioned into cyclohexane, and analyzed using a Hewlett Packard (HP) GC connected to an HP mass spectrometer for structural elucidation.

Radiolabeled Standards: The purity of [^{14}C]MSMA and [^{14}C]CA (cacodylic acid) analytical standards was adequately documented.

Identification of Radioactive Residues

The aqueous extract of cottonseed, containing 56% of the seed TRR, contained 26.5% as MSMA, 5.2% as CA, 3.2% as Unknown 1, and 21.1% as Unknown 2. The retention time for Unknown 1 was 3.5 minutes, and that of Unknown 2, 33.5 min. The MeOH extract, with a total of 5% of the seed TRR, contained 1.2% as MSMA and 0.8% as CA; Unknown 2 was present, but not at a concentration sufficient for quantitation. Adequate examples of HPLC radiochromatograms, as well as confirmatory TLC/autoradiograms for the aqueous and MeOH extracts were submitted. The hexane extract, the cottonseed oil fraction, was immiscible with the HPLC mobile phase; evaporation of the hexane to yield the oil alone produced the same result. Consequently, TLC alone was used to analyze the oil extract. While the report stated

that autoradiography and TLC scanning detected non-polar radioactivity, smeared up the plate from the origin, the chromatogram was not submitted for examination by CBRS. However, this should not be a problem, since the hexane fraction contained a relatively small amount of the seed TRR. It was postulated that the smear of non-polar radioactivity indicated incorporation of ^{14}C into endogenous plant compounds. An attempt was made to determine the presence of arsenic in the oil extract using graphite furnace/atomic absorption spectroscopy, but the results were inconclusive due to the potential for matrix interferences.

The aqueous portion of the acid hydrolysate contained 9% of the cottonseed TRR, and consisted of 3.8% Unknown 1, 3.3% Unknown 2, and 1.9% Unknown 3 (retention time, 17.5 minutes). The level of radioactivity present in the organic extract of the hydrolysate (1% of the seed TRR) was insufficient for analysis/characterization. The aqueous portion of the base hydrolysate contained 5% of the seed TRR, and consisted of 3.2% Unknown 1, and 1.8% Unknown 4. The shoulder on the Unknown 1 peak, located at 5 minutes RT, was considered to be an artifact of the fractionation system. This conclusion seems reasonable, since a considerable amount of amplification was necessary, due to the low level of radioactivity (0.07 ppm) present in the extract. Unknown 4 had a retention time of 38.5 minutes. An adequate HPLC radiochromatogram for the aqueous phase of the base hydrolysate was submitted for CBRS review. HPLC analysis of the organic fraction of the base hydrolysate revealed the presence of 5 additional unknowns, all at levels of <0.01 ppm; characterization of these peaks was not attempted, nor were supporting chromatograms submitted.

The identification of MSMA and CA in the cottonseed aqueous extract was confirmed using GC/MS; total ion chromatograms (TICs), as well as spectra for the derivatized standards were presented in the study report. The TIC and spectrum for the cottonseed aqueous extract were also submitted, and appear to support the identification of MSMA and CA in the extract. Efforts to identify radioactive residues were concentrated on the aqueous extract, since it contained 56% of the cottonseed TRR. Unknowns 1 and 2 comprised the most significant portion of the unidentified radioactivity. Attempts to isolate these components were unsuccessful.

According to the study report, ammonium hydroxide (NH_4OH) extracts of soil spiked with [^{14}C]MSMA and [^{14}C]CA contained two unknown components having retention times of 3 and 34 minutes, which were the same as Unknowns 1 and 2 found in the aqueous extract of cottonseed. Soil extracts were fractionated using HPLC, the peaks at 3 and 34 minutes collected, and the radioactivity further analyzed using HPLC, TLC, and GC/MS; however, the initial HPLC radiochromatograms from the soil extracts were not submitted. The initial soil radiochromatograms must be submitted, in order to confirm that Unknowns 1 and 2 identified in the cottonseed aqueous extracts are the same as those identified in the soil. In addition, a brief description of the soil conditions and fortifications and the extraction procedure should be submitted.

Re-injection of the fraction containing the metabolite represented by the peak designated Unknown 1 into the same HPLC system resulted in a broad peak which eluted at 15 minutes.

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The report stated that although no distinct peak was visible, this broad peak at 15 minutes included the peak for MSMA at 12 minutes. Analysis of the peak using TLC and autoradiography demonstrated that Unknown 1 co-migrated with radiolabelled MSMA. HPLC and TLC chromatograms were submitted in support of these conclusions. Re-injection of the peak observed at 34 minutes into the same HPLC system resulted in a broad and poorly resolved peak at 12 minutes. It was concluded that this peak also contained the peak for MSMA, and a TLC chromatogram supporting this conclusion was submitted, but the radioactive band was poorly resolved. The isolation of these totally different peaks from the same system was explained as an artifact of the HPLC system, which relies on ion-pairing of analytes with an alkylamine agent. CBRS has no basis to agree with this interpretation; there is no way for CBRS to verify that the 34-minute peak from the soil was the same as the 12-minute re-injected peak.

Attempts to derivatize and analyze Unknown 1 using GC/MS were unsuccessful; however, the thiomethylglycolate derivative of Unknown 2 was formed, and analyzed using GC/MS, confirming the presence of MSMA. Since the GC/MS data support the identification of a moiety containing MSMA in Unknown 2, no additional analytical work will be required at this time. However, since Unknown 2 comprises almost 25% of the unidentified cottonseed TRR, the registrant is required to submit a more detailed summary of the soil extract analytical work. The registrant must submit a detailed discussion explaining why the retention time for Unknown 2 changed so dramatically within the same chromatographic system. Unknown 1 accounted for 10% of the cottonseed TRR; at this time, CBRS will not require that Unknown 1 be further characterized. However, a final decision as to whether additional soil analytical work is required will be made pending receipt of the additional information regarding soil extraction and analysis.

The intent of the tolerance is to regulate all arsenic-containing metabolites of concern. Consequently, the registrant is required to analyze the cottonseed for total arsenic (e.g. using atomic absorption methods), and compare the results to the cottonseed TRR. Levels of arsenic in the radioactive sample could also be compared with results of the radiovalidation, using data collection and enforcement methods. The results of such analyses could confirm that all of the ^{14}C in cottonseed exists associated with As.

Table 2. HPLC Identification of Radioactivity in Aqueous and Organic Extracts of Cottonseed[a]

Extract[b]	% TRR in Extract (ppm)[c]	Compound		Unknown			
		MSMA (9.5 - 10.0)[d]	CA (6.0)	1 (2.5)	2 (34.5)	3 (17.5)	4 (38.5)
H_2O	56 (0.83)	26.5 (0.39)	5.2 (0.08)	3.2 (0.05)	21.1 (0.31)	nd (nd)[e]	nd (nd)
MeOH	2 (0.03)	1.2 (0.02)	0.8 (0.01)	nd (nd)	nd (nd)	nd (nd)	nd (nd)

Table 2. HPLC Identification of Radioactivity in Aqueous and Organic Extracts of Cottonseed[a]

Extract[b]	% TRR in Extract (ppm)[c]	Compound		Unknown			
		MSMA (9.5 - 10.0)[d]	CA (6.0)	1 (2.5)	2 (34.5)	3 (17.5)	4 (38.5)
Aqueous of Acid Hydrolysate	9 (0.13)	nd (nd)	nd (nd)	3.8 (0.06)	3.3 (0.05)	1.9 (0.03)	nd (nd)
Aqueous of Base Hydrolysate	5 (0.07)	nd (nd)	nd (nd)	3.2 (0.05)	nd (nd)	nd (nd)	1.8 (0.03)
Total	72 (1.06)	27.7 (0.41)	6.0 (0.09)	10.2 (0.16)	26.2 (0.36)	1.9 (0.03)	1.8 (0.03)

[a] Radioactive residues are presented as follows: % of the cottonseed TRR (ppm radioactivity).

[b] The residues in the hexane fraction constituted 5% of the cottonseed TRR, and could not be characterized using HPLC; TLC analysis resulted in a smear of radioactivity up the plate, indicating possible incorporation into endogenous plant compounds. Residues in the organic extracts of the acid and base hydrolysates contained too little radioactivity for characterization.

[c] %TRR refers to the cottonseed TRR only.

[d] The values in parentheses indicate HPLC retention times (minutes); the HPLC retention time for MSMA ranges from 9.5 to 10.0 minutes.

[e] nd = none detected.

Additional Comments:

Both the greenhouse and analytical phases of the study were conducted according to the GLP standards specified in 40 CFR, part 160. The study report did not include validation data for data collection or enforcement methods using cottonseed generated in the metabolism study. Cottonseed should be analyzed by these methods, the results compared to the TRR, and the identifications made in the metabolism study confirmed.

Citrus (Lemon) Metabolism Study: MRID Nos. 42324401 and 42391201

Reviewer's Note: MRID No. 42391201 contained corrections to MRID No. 42324401 that were made following the inclusion of final QA audit comments in the study. The changes made were all minor, and did not affect the outcome of the metabolism study.

Radiolabelled Test Substance and Application to Lemon Trees

Monosodium methanearsonate (MSMA) was labelled with ^{14}C in the methyl group; the radiolabelled material was obtained from Ricera, Inc. The specific activity of the [^{14}C]MSMA was 2.4 mCi/mmole, with a radiochemical purity of 99.6%, as determined by HPLC. Adequate supporting data were submitted. The specific activity of the treatment solution applied to lemon trees was 2.4 mCi/mmole.

Meyer lemon trees 2 to 3 years old (2-4 ft. high), grown in 5 gallon containers, were acclimatized to the conditions of the test site for 30 days prior to initiation of the treatment program. A total of 7 treated and 4 control trees were maintained in separate screenhouses; the control screenhouse was located upwind from the ^{14}C screenhouse. Shade cloth and plastic were used to protect the trees from the elements during the study. The physical characteristics of the sandy loam soil were adequately described. Irrigation, fertilization weather conditions were adequately described.

The control application solution consisted of water and surfactant (Triton X-100). The ^{14}C treatment solution consisted of ^{14}C-MSMA and surfactant. Lemon trees received three applications between 8/21/90 and 11/5/90; registered labels allow up to 3 applications per year. Pipets were used to apply the treatment and control solutions to the soil in concentric circles around the tree trunks; care was taken to avoid contact with the leaves and trunks. Lemon trees were treated with 3 times with MSMA at an exaggerated rate (4.86 lb a.i./A) to simulate the maximum rate allowed for disodium methanearsonate (DSMA) on the Drexel 81P label [EPA Reg. No. 19713-113], which calls for applications to bearing and nonbearing trees (except in FL). Each tree was treated three times with 54 - 58 mg ^{14}C MSMA, or a total of approximately 172 mg/pot (mg/tree) during the course of the study. The 3 applications were made 104, 59, and 28 days PHI.

A preliminary harvest of one lemon and a few leaves from ^{14}C-treated trees was made immediately following the 3rd application. Some of the trees had immature fruits at the time the first application was made; at final harvest (12/3/90, 28 days following the final application), 90% of the lemons were mature (yellow), while 10% were light green to yellow in color. Fruit and leaf samples were placed in bags and frozen on dry ice. Samples were shipped frozen to PTRL East on 12/11/90 (no documentation for this shipment was provided).

The TRR in each sample was determined by combustion to $^{14}CO_2$ followed by liquid scintillation counting (LSC). The limit of quantitation (LOQ) for the combustion assay was 0.003 ppm for lemon pulp and 0.01 ppm for peel, leaves and juice. Determination of the TRR for lemon tree components was based on the wet weight of the samples. Lemon peel contained 0.44 ppm of radioactivity (53% of the fruit TRR); lemon pulp contained 0.07 ppm of radioactivity (24% of the TRR); and lemon juice contained 0.12 ppm (23% of the TRR).

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Extraction and Hydrolysis Procedure

Lemons were manually separated into peel, pulp, seed and juice samples. Juice was obtained by squeezing the peeled fruit, and the remaining solids were classified as pulp. Lemon peel and pulp samples were extracted first either by stirring with water for 4 hours, or by homogenization with water for 2 - 4 minutes, filtered, and the process repeated 2 times. Water filtrates were combined and set aside for analysis. The remaining solids were extracted in a similar manner, first with methanol (MeOH), and then with hexane. In each case, solids were extracted 3 times, and the filtrates combined for analysis. The hexane fractions were evaporated to remove the solvent, leaving lemon oil. Juice was not extracted, but analyzed directly. No acid or base hydrolysis steps were required to release additional radioactivity. A summary of the radioactivity found in each of the fractions is presented in Table 3.

Table 3. Distribution of Radioactive Residues in Lemon Extracts[a]

	Solvent Extract								Method	
	H_2O		MeOH		Hexane[b]		Bound		Recovery[c]	
Sample Matrix	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm
Lemon Peel	94	0.42	2	0.01	1	0.01	3	0.01	100	0.44
Lemon Pulp	97	0.07	9	0.01	6	<0.01	0	<0.01	112	0.08
Lemon Juice[d]	100	0.12	--	--	--	--	--	--	100	0.12

[a] % = ppm radioactivity in the fraction/ppm radioactivity in the matrix (i.e. peel, pulp, and juice); ppm = ppm radioactivity in the fraction.

[b] The hexane fraction includes lemon oil.

[c] Method recovery is the total radioactivity in all fractions divided by the total radioactivity in the matrix, determined by combustion.

[d] Radioactivity in lemon juice was determined directly (no extraction needed).

Analytical Techniques used for Characterization and Identification of Radioactive Residues

Lemon extracts were analyzed using HPLC, in order to identify or characterize radioactive residues. The identities of radiolabelled components separated by HPLC were confirmed using TLC. Confirmation of the identification of MSMA and CA (cacodylic acid) in lemons was accomplished by derivatization, followed by GC/MS analysis. Each of these systems has been described in detail within the summary of the cottonseed metabolism study (see above).

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Identification of Radioactive Residues

The lemon peel aqueous extract contained 18.1% of the lemon peel TRR as MSMA, 54% as cacodylic acid (CA), and 21.5% as Unknown 1 (corresponding to Unknown 1 identified in the cottonseed metabolism study). The organic extract contained 1% of the lemon peel TRR as MSMA, 0.4% as CA, 0.2% as Unknown 1, and 0.5% as Unknown 3.

The lemon pulp aqueous extract contained 10% of the pulp TRR as MSMA, 61.2% as CA, and 25.8% as Unknown 1. The organic extract of the lemon pulp contained insufficient radioactivity to warrant further attempts at characterization.

Hexane extracts of lemon peel and pulp were immiscible with the HPLC mobile phase; evaporation of the hexane yielded lemon oil, which was also immiscible with the mobile phase. However, since the combined hexane extracts contained only approximately 0.01 ppm of radioactivity, further chromatographic analysis was not attempted.

Lemon juice contained 13.3% of the juice TRR as MSMA, 51.8% as CA, 27% as Unknown 1, and 7.9% as Unknown 2.

Adequate supporting documentation/chromatograms were included in the study report. A summary of the radioactive residues identified in lemon peel, pulp, and juice is presented in Table 4.

Table 4. Identification of Radioactive Residues in Lemon Matrices[1]

Lemon Fraction	MSMA	Cacodylic Acid	Unknown 1[2]	Unknown 2	Unknown 3
Peel	19.1 (0.09)	54.7 (0.24)	21.7 (0.10)	ND[3]	0.5 (<0.01)
Pulp	10.0 (0.01)	61.2 (0.04)	25.8 (0.02)	ND	ND
Juice	13.3 (0.02)	51.8 (0.06)	27.0 (0.03)	7.9 (0.01)	ND

[1] Residues are presented as % TRR (ppm radioactivity).

[2] Unknown 1 was shown to contain MSMA in the cottonseed metabolism study.

[3] ND = Not Detected.

While similar Unknowns (unidentified metabolites) were found in both the cottonseed and citrus metabolism studies, there were qualitative differences. Unknown 3 did not constitute a significant percentage of the TRR in either study; Unknown 4 was not a significant residue in the cottonseed metabolism study, but was not identified at all in the lemon metabolism study. Unknowns 1 and 2 represented significant percentages of the radioactivity in both studies;

however, Unknown 2 contained 26% of the cottonseed TRR, but was not present in the peel or pulp, and comprised only 8% of the lemon juice TRR. While Unknown 1 comprised 10% of the cottonseed TRR, it was present as nearly 22% of the peel TRR, 26% of the pulp TRR, and 27% of the juice TRR (an average of 25% of the lemon TRR). In the cottonseed study, Unknown 1 was successfully derivatized, and shown to contain MSMA.

Since Unknown 2 comprised only 7.9% of the unidentified radioactivity in lemon juice (0.01 ppm), and was not found at all in peel and pulp, it is not considered to be a significant residue in lemons. Consequently, CBRS will not require that additional analytical work be done for the purpose of this metabolism study. CBRS concludes that the residues of concern in lemons consist of MSMA and cacodylic acid; although cacodylic acid residues comprised more of the TRR, both MSMA and cacodylic acid residues can be considered to be equally significant.

cc: CBSwartz; MSMA List B file; RF; SF; Circulation
H7509C:CBRS:CBSwartz:CM#2:Rm 800D:703 305 5877:4/15/92
RDI:WJHazel:5/8/92 MSMetzger:5/25/93 EZager:5/27/93