MEMORANDUM

SUBJECT: Acephate. List A Case No. 0042. Chemical No. 103301. Registant's Response to Residue Chemistry Data Requirements. CBRS Nos. 17188, 17189, 17190, 17191, 17427, 17429. DP Barcodes D225794, D225795, D225796, D225786, D228007, D227969 .

FROM: Felecia A. Fort, Chemist
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THRU: Randolph B. Perfetti, Ph.D., Acting Branch Chief
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TO: Paula Deschamp, Section Head
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Attached is a review of plant and animal metabolism, mint hay field trials, peanut processing and storage stability studies, and analytical methods. This information was reviewed by Dynamac Corporation under the supervision of CBRS/HED. The data assessment has undergone secondary review in the Branch and has been revised to reflect Agency policies.

CBRS makes the following conclusions with respect to the submitted study:

> The nature of the residue in plants and animals is adequately understood pending submission of additional storage stability data. The residues of concern are acephate and methamidiphos.
>
> ***NOTE:*** The Agency intends to modify the tolerance expression for acephate to express tolerances in terms of acephate *per se*, and to list tolerances for methamidophos residues resulting from application of acephate with the tolerances for methamidophos residues

resulting from application of methamidophos [under 40 CFR §180.315] to be consistent with Codex. These issues will be addressed at the issuance of the RED.

The submitted supplemental storage stability data are acceptable and indicate that fortified residues of acephate and its metabolite methamidophos are relatively stable under frozen storage conditions (-20 C) for at least 4 months in/on peanut oil.

. The submitted data indicate that residues of acephate and methamidophos will not exceed the established tolerances of 15 ppm (for combined residues of acephate and methamidophos) and 1 ppm (for methamidophos) in/on mint hay harvested 14 days following two applications of the 75 SC formulation at 1.0 lb ai/A/application (1x the maximum seasonal rate) using aerial equipment. Residues of acephate were 1.9-11 ppm and residues of methamidophos were 0.33-1.1 ppm in/on samples collected 14 days following one application to first-growth mint and one application to second-growth mint or following two applications to first-growth mint.

Based on previously submitted data (MRID 40508503; reviewed in the Update) for fresh mint hay and the current study, the registrant must propose higher tolerances for residues of acephate and methamidophos in/on mint tops (fresh mint hay) at 27 ppm and 2 ppm, respectively. Increased tolerances may not be required if the registrant can provide an adequate explanation for the previously reported tolerance-exceeding residues.

The submitted peanut processing data are adequate and indicate that residues of acephate and its metabolite methamidophos do not concentrate in refined oil processed from peanuts with detectable residues. However, the data indicate that residues of acephate and methamidophos may concentrate in peanut meal at 2x and 3x, respectively. The maximum expected acephate and methamidophos residues in peanut meal would be 0.16 and 0.06 ppm, respectively. Since the expected residues in peanut meal do not exceed the recommended individual tolerances for acephate and methamidophos for the RAC (0.2 and 0.1 ppm, respectively), no tolerances for peanut meal are required.

cc: Reviewer(F. Fort), *B. Lavis-Sjoblad\L. Schnaubelt (SRRD)*, Reg. Std. File, RF, SF, Circ.
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Disk8:acephate.

ACEPHATE

Shaughnessy No. 103301; Case 0042
(CBRS No. 17188, DP Barcode D225794;
CBRS No. 17189, DP Barcode D225795;
CBRS No. 17190, DP Barcode D225796;
CBRS No. 17191, DP Barcode D225786;
CBRS No. 17427, DP Barcode D228007;
CBRS No. 17429, DP Barcode D227969)

Registrant's Response to Residue Chemistry Data Requirements

January 22, 1997

Contract No. 68-D4-0010

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
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ACEPHATE

Shaughnessy No. 103301; Case 0042

(CBRS No. 17188, DP Barcode D225794; CBRS No. 17189, DP Barcode D225795; CBRS No. 17190, DP Barcode D225796; CBRS No. 17191, DP Barcode D225786; CBRS No. 17427, DP Barcode D228007; CBRS No. 17429, DP Barcode D227969)

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

In response to the Acephate Reregistration Standard Update, dated 1/29/92, Valent U.S.A. Corporation has submitted data depicting the metabolism of acephate in beans (1996; MRID 43971603), cotton (1996; MRID 44037801), lettuce (1996; MRID 43971602), lactating goats (1996; MRID 43971604), and laying hens (1996; MRIDs 43971605 and 44037803). Valent has also submitted radiovalidation data for beans (1996; MRID 43971606), cotton (1996; MRID 44037802), lettuce (1996; MRID 43971607), milk and liver (1996; MRID 43971608), and eggs and muscle (1996; MRIDs 43971609 and 44307804), field trial data for mint hay (1995; MRID 43971610), and a processing study for peanuts (1995; MRID 43971611). Data from these submissions are evaluated herein for adequacy in fulfilling residue chemistry data requirements for the reregistration of acephate. We note that MRIDs 44037803 and 44037804 are amended reports for MRIDs 43971605 and 43971609, respectively; therefore, MRIDs 43971605 and 43971609 will not be reviewed here. The Conclusions and Recommendations stated below pertain only to the above submissions. All other residue chemistry data requirements stated in the Acephate Update are not addressed herein.

Tolerances have been established for the combined residues of acephate (O,S-dimethyl acetylphosphoramidothioate) and its cholinesterase-inhibiting metabolite O,S-dimethyl-phosphoramidothioate (methamidophos) in/on various raw agricultural and processed plant and animal commodities ranging from 0.02 ppm for food items as a result of use of acephate in food-handling establishments to 15 ppm for grass forage and hay and mint hay [40 CFR §180.108(a) and (b), §185.100, and §186.100]. Tolerances for several commodities (beans, Brussels sprouts, cauliflower, celery, cranberries, lettuce, mint hay, and peppers) include limits on residues of methamidophos. The Agency intends to modify the tolerance expression for acephate to express tolerances in terms of acephate *per se*, and to list tolerances for methamidophos residues resulting from application of acephate with the tolerances for methamidophos residues resulting from application of methamidophos [under 40 CFR §180.315] (see W.T. Chin, 6/23/86, CB No. 942).

Adequate methods are available for the enforcement of established tolerances. The Pesticide Analytical Manual (PAM) Volume II lists Methods I and II, GLC methods employing thermionic detection, as well as Method A, a confirmatory TLC method. Codex MRLs have been established for residues of acephate *per se*. Separate Codex MRLs have been established for residues of methamidophos *per se*. Issues pertaining to compatibility of U.S. tolerances with Codex MRLs will be addressed at the issuance of the RED.

CONCLUSIONS AND RECOMMENDATIONS

Qualitative Nature of the Residue in Plants

1. The qualitative nature of the residue in plants is adequately understood pending submission of additional information and data pertaining to storage stability. The residues of concerns are acephate and methamidiphos. For the bean and lettuce metabolism studies, the registrant must submit information regarding the dates of analysis of samples to allow CBRS to determine that the submitted storage stability data are adequate to support the metabolism studies. For the cotton metabolism study, the registrant must supply the dates of analysis of samples, and provide data indicating that the metabolite profile in cotton commodities did not change over the intervals for which samples were stored.

2a. Beans: Total radioactive residues (TRR) were 16.216 ppm or 12.385 ppm in whole beans and 74.433 ppm or 85.278 ppm in bean forage collected 14 days following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate, respectively, at 1 lb ai/A/application (1x the maximum registered single application rate).

2b. In beans from the S-methyl treatment, ~66 of TRR in whole beans and ~110 of TRR in forage was characterized/identified. Acephate was identified at 13.7 of TRR (2.236 ppm) in whole beans and 74.4 of TRR (55.377 ppm) in bean forage. The metabolites

methamidophos and S-methyl N-acetylphosphoramidothioate (SMPT) were identified at 7.3 of TRR (1.187 ppm) and 8.6 of TRR (1.388 ppm), respectively, in whole beans, and at 7.6 of TRR (5.692 ppm) and 14.2 of TRR (10.549 ppm), respectively, in forage. The full chemical names and chemical structures of identified metabolites are presented in Figure 1.

2c. In beans from the carbonyl treatment, ~90 of TRR in whole beans and ~101 of TRR in forage was characterized/identified. Acephate was identified at 14.8 of TRR (1.834 ppm) in whole beans and 62.5 of TRR (53.313 ppm) in bean forage. The metabolites O-methyl N-acetylphosphoramidate (OMAPAA) and SMPT were identified at 56.6 of TRR (7.013 ppm) and 7.0 of TRR (0.869 ppm), respectively, in whole beans, and at 22.5 of TRR (19.171 ppm) and 6.5 of TRR (5.547 ppm), respectively, in forage.

3a. Cotton: Total radioactive residues were 3.100 ppm or 0.511 ppm in cotton seed meal, 2.043 ppm or 0.462 ppm in cotton seed hulls, and 12.756 ppm or 13.225 ppm in gin trash collected 21 days following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate, respectively, at 1 lb ai/A/application (1x the maximum single application rate). The registrant attributed the higher TRR in S-methyl-treated seed meal and hulls more rapid cleavage of the S-methyl group, which resulted in more rapid incorporation into plant parts.

3b. In cotton commodities from the S-methyl treatment, ~85-89 of TRR was characterized/identified. Acephate was identified at 0.8 of TRR (0.025 ppm) in seed meal, 2.1 of TRR (0.043 ppm) in seed hulls, and 37.7 of TRR (4.821 ppm) in gin trash. Methamidophos was identified at 0.5 of TRR (0.014 ppm) and 1.5 of TRR (0.189 ppm), respectively, in seed meal and gin trash. The metabolites SMPT and S-methyl phosphoramidothioate (SMPAA) were identified at 3.0 of TRR (0.096 ppm) and 0.9 of TRR (0.029 ppm), respectively, in seed meal, at 3.9 of TRR (0.079 ppm) and 0.7 of TRR (0.015 ppm), respectively, in seed hulls, and at 26.4 of TRR (3.374 ppm) and 0.8 of TRR (0.108 ppm), respectively, in gin trash. The metabolite O,S-dimethyl phosphorothioate (DMPT) was identified in gin trash only at 4.0 of TRR (0.503 ppm). In addition, a lipid component characterized as triglycerides comprised 35.2 of TRR (1.091 ppm) in seed meal and 12.5 of TRR (0.256 ppm) in seed hulls.

3c. In cotton commodities from the carbonyl treatment, ~92-94 of TRR was characterized/identified. Acephate was identified at 2.0 of TRR (0.010 ppm) in seed meal, 7.1 of TRR (0.033 ppm) in seed hulls, and 37.3 of TRR (4.928 ppm) in gin trash. The metabolites SMPT, OMAPAA, and acetamide were identified at 1.0 of TRR (0.005 ppm), 21.8 of TRR (0.111 ppm), and 2.5 of TRR (0.013 ppm), respectively, in seed meal, at 2.4 of TRR (0.011 ppm), 23.6 of TRR (0.109 ppm), and 9.3 of TRR (0.043 ppm), respectively, in seed hulls, and at 16.0 of TRR (2.118 ppm), 24.9 of TRR (3.293 ppm), and 1.6 of TRR (0.205 ppm), respectively, in gin trash. In addition, a lipid component

characterized as triglycerides comprised 20.0 of TRR (0.102 ppm) in seed meal and 3.7 of TRR (0.017 ppm) in seed hulls.

4a. Lettuce: Total radioactive residues were 3.135 ppm or 1.689 ppm in lettuce collected 21 days following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate, respectively, at 1 lb ai/A/application for a total application rate of 3 lb ai/A (~0.6x the maximum seasonal rate).

4b. In lettuce from the S-methyl treatment, ~85 of TRR was characterized/identified. Acephate was identified at 39.8 of TRR (1.248 ppm), the metabolites methamidophos and SMPT were identified at 8.7 of TRR (0.271 ppm) and 8.4 of TRR (0.262 ppm), respectively. An unknown characterized as a neutral natural product comprised 18 of TRR (0.564 ppm).

4c. In lettuce from the carbonyl treatment, ~83 of TRR was characterized/identified. Acephate was identified at 35.6 of TRR (0.601 ppm), the metabolites OMAPAA and SMPT were identified at 26.4 of TRR (0.446 ppm) and 11.5 of TRR (0.195 ppm), respectively.

5. Radiovalidation of the enforcement method: The submitted radiovalidation data for enforcement method RM-12A-9 are adequate. The method adequately recovered residues of acephate and methamidophos from samples of beans and lettuce treated with [S-methyl-^{14}C]acephate (from the bean and lettuce metabolism studies). In addition, the method adequately recovered residues of acephate at levels near the LOQ from samples of cottonseed meal treated with [S-methyl-^{14}C]acephate (from the cotton metabolism study); residues of methamidophos were below the LOQ in the sample, and were not detected.

Qualitative Nature of the Residue in Animals

6. The qualitative nature of the residue in animals is adequately understood pending submission of additional data pertaining to storage stability. The residues of concern are acephate and methamidiphos. The registrant must submit data demonstrating that the metabolic profile of radioactive residues in poultry muscle did not change significantly between the initial analysis (completed within 35 days of collection) and the repeat analyses (conducted ~15 months later).

7a. Goats: Following oral administration of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate to lactating goats for 3 days at 15 ppm (~0.4x the maximum dietary burden), the TRR, respectively, were 0.2349-0.5769 ppm and 0.2074-0.7362 ppm in milk, 0.018 ppm and 0.103 ppm in fat, 0.168 ppm and 0.200 ppm in muscle, 0.502 ppm and 0.358 ppm in kidney, and 1.085 ppm and 1.042 ppm in liver.

7b. The majority of ^{14}C-residues, ~70-98 , were characterized/identified in milk, fat, muscle, kidney, and liver. Following dosing with the S-methyl-labeled test substance, acephate was identified in milk (14.6 TRR, 0.077 ppm), muscle (26.2 TRR, 0.044 ppm), kidney (13.9 TRR, 0.070 ppm), and liver (4.2 TRR, 0.046 ppm). Methamidophos was identified in milk (1.0 TRR, 0.005 ppm) and was detected in muscle, kidney, and liver at levels <0.01 ppm. Other identified metabolites included SMPT (7.0 TRR, 0.037 ppm in milk; and 0.6 TRR, 0.006 ppm in liver), and SMPAA (5.6 TRR, 0.028 ppm in kidney; and 1.3 TRR, 0.014 ppm in liver). The registrant demonstrated the incorporation of radioactivity into natural products by identifying [^{14}C]lactose (32.9 TRR, 0.174 ppm) in milk and by showing that 25.1 of liver TRR (0.272 ppm) consisted of high molecular weight compounds (>12,000 amu). No metabolites were identified in fat; however, TRR in fat were low (0.018 ppm).

7c. Following dosing with the carbonyl-labeled test substance, acephate was identified in milk (11.6 TRR, 0.078 ppm), muscle (21.5 TRR, 0.043 ppm), kidney (25.7 TRR, 0.092 ppm), and liver (4.8 TRR, 0.050 ppm). Acetamide was identified in milk (3.3 TRR, 0.022 ppm) and liver (4.5 TRR, 0.047 ppm). Other identified metabolites included SMPT (1.2 TRR, 0.009 ppm in milk plus an additional 4.7 TRR, 0.032 ppm which was either SMPT or OMPT; 1.7 TRR, 0.006 ppm in kidney; and 2.5 TRR, 0.026 ppm in liver), and OMAPAA (10.3 TRR, 0.037 ppm in kidney; and 6.9 TRR, 0.072 ppm in liver). The registrant demonstrated the incorporation of radioactivity into natural products by identifying [^{14}C]lactose (4.0 TRR, 0.027 ppm) in milk, by identifying [^{14}C]capric acid and [^{14}C]palmitic acid in cream, and by showing that 26.8 of liver TRR (0.279 ppm) consisted of high molecular weight compounds (>12,000 amu). No metabolites were identified in fat; however, HPLC analyses indicated radioactivity eluting at the retention times of capric, myristic, and palmitic acids.

8a. Poultry: Following oral administration of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate to laying hens for 3 days at 10 ppm (~5x the maximum dietary burden), the TRR, respectively, were 0.08-0.34 ppm and 0.02-0.31 ppm in egg white, 0.02-0.17 ppm and <0.021-1.52 ppm in egg yolk, 0.04 ppm and 0.44 ppm in fat, 0.10 ppm and 0.11 ppm in muscle, and 0.46 ppm and 0.87 ppm in liver.

8b. The majority of ^{14}C-residues, ~61->100 , were characterized/identified in egg white, egg yolk, fat, and liver of hens following dosing with the S-methyl-labeled test substance; only 24 of ^{14}C-residues in muscle were characterized/identified. Acephate was identified in egg white (42.4 TRR, 0.144 ppm), egg yolk (32.6 TRR, 0.056 ppm), fat (25.7 TRR, 0.010 ppm), muscle (9.9 TRR, 0.010 ppm), and liver (11.0 TRR, 0.051 ppm). Methamidophos was identified in egg white (9.5 TRR, 0.032 ppm), fat (2.7 TRR, 0.001 ppm), and muscle (13.6 TRR, 0.013 ppm). Other identified metabolites were SMPT (3.3-17.0 TRR, 0.006-0.054 ppm), DMPT (0.9-1.7 TRR, <0.001-0.006 ppm), and SMPAA (0.7-5.9 TRR, 0.001-0.027 ppm); these metabolites were each detected in egg

white, egg yolk, fat, and liver. The registrant demonstrated the incorporation of radioactivity into glutamic acid in egg white. One unknown (P1) which detected in egg white at 17.9 TRR (0.061 ppm) was characterized to be a polar natural product based on its HPLC peak shape and UV spectrum. Another unknown, detected in liver at 9.3 TRR (0.043 ppm), was not identified because of its low residue level.

8c. The majority of ^{14}C-residues, ~72->100 , were characterized/identified in egg white, egg yolk, fat, muscle, and liver following dosing with the carbonyl-labeled test substance. Acephate was identified in egg white (61.7 TRR, 0.190 ppm), egg yolk (5.3 TRR, 0.080 ppm), fat (0.7 TRR, 0.003 ppm), muscle (40.8 TRR, 0.045 ppm), and liver (2.4 TRR, 0.021 ppm). Acetamide was identified in egg white (10.5 TRR, 0.032 ppm), egg yolk (1.0 TRR, 0.016 ppm), fat (0.6 TRR, 0.003 ppm), muscle (10.0 TRR, 0.011 ppm), and liver (7.9 TRR, 0.069 ppm). Other identified metabolites were SMPT (all matrices, 0.7-4.2 TRR, 0.001-0.014 ppm) and OMAPAA (all matrices except egg white, 0.2-4.2 TRR, 0.001-0.037 ppm). The registrant demonstrated the incorporation of radioactivity into fatty acids (palmitic and oleic acid) in egg yolks; 49.4 of egg yolk TRR (0.75 ppm) was saponifiable.

9. Radiovalidation of the enforcement method: The submitted radiovalidation data for enforcement method RM-12A-9 are adequate. The method adequately recovered residues of acephate from samples of milk, goat liver, egg white, and muscle from goats and hens treated with [S-methyl-^{14}C]acephate (from the metabolism studies). Although the recovery of methamidophos from these same samples was low, levels of methamidophos were near to or below the method limit of quantitation in the samples.

10. CBRS in consultation with Toxicology Branch II (Alberto Protzel) has determined that the metabolites, OMAPAA, SMPT, SMPAA, and DMPT are not of concern. The residues to be regulated in plants and animals are acephate and methamidiphos.

Storage Stability Data

11a. The submitted supplemental storage stability data are acceptable and may be used to partially satisfy reregistration requirements for this GLN topic. The data indicate that fortified residues of acephate and its metabolite methamidophos are relatively stable under frozen storage conditions (-20 C) for at least 4 months in/on peanut oil.

11b. A determination regarding the adequacy of available storage stability data to support the storage intervals and conditions of samples from the submitted mint hay field trial and peanut processing studies will be made when MRIDs 41081601 and 44055201 have been reviewed by CBRS.

Magnitude of the Residue in Mint Hay

12a. The submitted data indicate that residues of acephate and methamidophos will not exceed the established tolerances of 15 ppm (for combined residues of acephate and methamidophos) and 1 ppm (for methamidophos) in/on mint hay harvested 14 days following two applications of the 75 SC formulation at 1.0 lb ai/A/application (1x the maximum seasonal rate) using aerial equipment. Residues of acephate were 1.9-11 ppm and residues of methamidophos were 0.33-1.1 ppm in/on samples collected 14 days following one application to first-growth mint and one application to second-growth mint or following two applications to first-growth mint.

12b. Previously submitted data (MRID 40508503; reviewed in the Update) for fresh mint hay indicated that the established tolerance for the combined residues of acephate and methamidophos was too low. Residues exceeded the tolerance in/on two samples of mint hay collected 14 days following a single application of the 75 SC formulation at 1 lb ai/A (0.5x the maximum seasonal rate) using aerial equipment; maximum residues of acephate were 26.2 ppm and maximum residues of methamidophos were 1.62 ppm. Based on these data, the Update required that the registrant modify the product labels to lengthen the PHI, reduce the maximum number of applications, or reduce the maximum single application rate, and submit supporting residue data. However, the current field trial data reflect the registered use pattern which has not been amended. The registrant must propose higher tolerances for residues of acephate and methamidophos in/on mint tops (fresh mint hay) at 27 ppm and 2 ppm, respectively. Increased tolerances may not be required if the registrant can provide an adequate explanation for the previously reported tolerance-exceeding residues.

12c. According to Table 1 (OPPTS 860.100), the RAC of peppermint and spearmint is "tops (leaves and stems)"; the previous livestock feeds table listed "hay" to be the RAC for mint. The Agency considers any tolerances for "mint hay" to also cover residues from "fresh market mint" or "bunched mint"; see memo by G. J. Herndon (CBTS No. 10276, DP Barcode D180904, 12/10/92). Appropriate changes in the RAC definition for mint, as stated in 40 CFR §180.108(a), will be made at issuance of the Acephate RED.

Magnitude of the Residue in Peanut Processed Commodities

13a. The submitted peanut processing data are adequate and indicate that residues of acephate and its metabolite methamidophos do not concentrate in refined oil processed from peanuts with detectable residues. However, the data indicate that residues of acephate and methamidophos may concentrate in peanut meal at 2x and 3x, respectively.

13b. Adequate peanut field trial data were presented in the Addendum to the Acephate Reregistration Standard dated 10/5/84. Combined residues of acephate and methamidophos were reported as 0.02-0.09 ppm (<0.01-0.02 ppm methamidophos) in/on

peanuts dug 14-52 days after 1-4 applications of the 75 SC formulation at 0.5-2.0 lb ai/A/application. Assuming a worst-case scenario, since individual acephate residue levels were not reported, the HAFT would be 0.08 ppm acephate (maximum combined residues of 0.09 ppm minus 0.01 ppm, the minimum methamidophos residues) and 0.02 ppm methamidophos. Based on these HAFTs, the maximum expected acephate and methamidophos residues in peanut meal would be 0.16 and 0.06 ppm, respectively. Since the expected residues in peanut meal do not exceed the recommended individual tolerances for acephate and methamidophos for the RAC (0.2 and 0.1 ppm, respectively), no tolerances for peanut meal are required.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Plants

Beans

Use patterns registered to Valent

The 75 SC formulations (EPA Reg. Nos. 59639-26 and 59639-89) are registered by Valent for multiple foliar applications to beans and lima beans (dry and succulent) at 0.25-1.0 lb ai/A/application at 7- to 10-day intervals. Applications may be made in a minimum of 20 gal/A of water when using ground equipment, and 2 gal/A of water when using aerial equipment. A 0-day PHI has been established for lima beans in succulent form and a 14-day PHI has been established for snap beans and dry beans. There is no maximum number of applications per growing season or maximum seasonal rate listed on product labels. The feeding of treated vines to livestock is prohibited.

In-life phase

Valent submitted data (1996; MRID 43971603) pertaining to the metabolism of [S-methyl-^{14}C]acephate and [carbonyl-^{14}C]acephate in beans. The in-life phase of the study was conducted at Plant Sciences, Inc. (Watsonville, CA). The test substances, [S-methyl-^{14}C]acephate (specific activity 52.1 mCi/mmol) and [carbonyl-^{14}C]acephate (specific activity 37.7 mCi/mmol), were isotopically diluted with unlabelled acephate and diluted with deionized water prior to application to give final specific activities of 3.66 mCi/mmol and 3.55 mCi/mmol, respectively; the radiochemical purity for both test substances was >96 . Beans in each of two treatment boxes received three foliar applications by hand trigger sprayer of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate at ~1 lb ai/A/application (1x the maximum single application rate) for a total application rate of ~3 lb ai/A. The first application was made when plants were at ~70 bloom, and the subsequent applications were made at 7-day retreatment intervals; mature beans and bean forage were harvested 14 days after final application. The registrant noted that the box containing control plants was placed close to the treatment box containing S-methyl-treated plants to monitor the uptake of radiolabeled volatile components released by treated plants. Adequate information pertaining to plant growth, maintenance, weather and screenhouse conditions, test material and application, and harvest was provided.

Mature beans were harvested by hand and were either separated into seeds and pods or retained as whole bean samples. Forage samples consisted of the remainder of the bean plants, including stalks, foliage, and immature pods. Collected samples were placed in plastic bags and were stored immediately on dry ice for shipment to the analytical laboratory, PTRL West, Inc. (Richmond, CA). All samples were shipped on the day of harvest, and were stored frozen (<0 C) at PTRL West until processing.

Total radioactive residues (TRR)

At PTRL West, samples of beans and forage were homogenized with dry ice and stored at ~-17 C prior to analysis. Triplicate aliquots of homogenized plant tissues were combusted and radioassayed by liquid scintillation counting (LSC). The limits of detection (LODs) were 0.0044 ppm for S-methyl-treated plants and 0.0042 ppm for carbonyl-treated plants. The TRR in samples of beans and forage are presented in Table 1.

Table 1. Total radioactive residues (TRR) in beans and forage following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate at 1.0 lb ai/A/application.

Matrix	TRR, ppm [^{14}C]acephate equivalents		
	S-Methyl	Carbonyl	Control [a]
Seeds	19.276	14.352	0.071
Pods	9.276	11.173	0.031
Whole beans	16.216	12.385	0.040
Forage	74.433	85.278	0.097

[a] **Control plants were placed near S-methyl-treated plants to monitor the uptake of radiolabeled volatile components released by treated plants.**

The registrant stated that the low levels of radioactivity detected in control beans and forage could be attributed to incorporation of $^{14}CO_2$ as a byproduct of metabolism from the S-methyl-treated bean plants. No further analysis was conducted on treated bean seeds and pods or on control samples.

Extraction and hydrolysis of residues

Homogenized whole bean and bean forage samples were subjected to extraction and hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Extracts were concentrated as necessary by rotary evaporation or by evaporation under a stream of nitrogen. The general extraction procedures are summarized below.

Samples of whole beans from each treatment were extracted three times with acetonitrile (ACN):water (1:1, v:v), once with water, and one additional time with ACN:water (1:1, v:v); after each extraction, the mixtures were centrifuged, and the resulting extracts were combined. Nonextractable residues were subsequently extracted with ACN:0.1 N HCl (1:1, v:v), and the

extract following centrifugation was reserved. The resulting nonextractable residues were refluxed for 3 hours with 6 N HCl then centrifuged, and the resulting supernatant was vacuum filtered. The same procedures were followed for forage, except that samples were initially extracted five times with ACN:water (1:1, v:v).

To determine the uptake of radioactivity into natural plant constituents, subsamples of beans and forage from both treatments were extracted five times with pH 7 potassium phosphate buffer and centrifuged, and the resulting nonextractable residues were subjected to the following sequential hydrolysis steps: starch digestion with α-amylase (0.05 M potassium phosphate buffer, pH 7, 30 C, 20 hours); protein digestion with pronase E (0.05 M Tris buffer, pH 7.2, 25 C, 20 hours); pectin digestion via mild acid hydrolysis (0.05 M sodium acetate:0.05 M EDTA (1:1, v:v), pH 4.5, room temperature for 60 hours, then 70 C for 3 hours); lignin digestion with glacial acetic acid and sodium chlorite (deionized water, 70 C, 4 hours); hemicellulose digestion with 24 aqueous potassium hydroxide (25 C, 24 hours) followed by 6 N acetic acid (25 C, 1 hour); and cellulose digestion with 72 sulfuric acid (25 C, 4 hours) followed by 24 potassium hydroxide (pH 6.5-7.5, until precipitated potassium sulfate dissolved).

The distribution of ^{14}C-activity in the extracts and hydrolysates of bean matrices is presented in Tables 2 and 3.

Characterization/identification of residues

Extracts and hydrolysates were analyzed by TLC and HPLC. Metabolites were identified by cochromatography with the following reference standards: acephate, methamidophos, S-methyl N-acetylphosphoramidothioate (SMPT), O-methyl N-acetylphosphoramidothioate (OMPT), O,S-dimethylphosphorothioate (DMPT), S-methylphosphoramidothioate (SMPAA), and O-methyl N-acetylphosphoramidate (OMAPAA). TLC identification of acephate and methamidophos was confirmed by GC/MS, and TLC identification of SMPT and OMAPAA was confirmed by ^{31}P-NMR following a separate extraction procedure.

The majority of TLC analyses were conducted on precoated silica gel (F254) plates; radioactivity was visualized using a radioscanner and/or by autoradiography, and quantitated by imaging software. Distinct radiolabelled bands detected by the radioscanner were scraped, dissolved in methanol and reapplied to new TLC plates. Nonlabeled components were visualized by placing plates in an iodine chamber, and subsequently spraying the plates with acidic palladium chloride. For analysis of polar material in various extracts, isolates from ACN:water extracts, and potassium phosphate buffer extracts, plates were developed with n-butanol:glacial acetic acid:water (6:1:1, v:v:v); for determination of ACN:water extracts and isolates, plates were developed with ACN:water:ammonium hydroxide (85:12:3, v:v:v). TLC analysis of carbohydrates for selected samples was conducted on hard-layer silica gel plates developed with n-butanol:acetone:water (4:5:1, v:v:v). Prior to development, the plates were impregnated with 0.3 M potassium

phosphate, and dried. Nonlabeled standards were visualized by spraying the plates with concentrated sulfuric acid, then heating.

Reverse-phase HPLC analysis was conducted using a Supelco C-18 column using a gradient mobile phase of deionized water and ACN. Nonlabeled standards were detected by UV (215 nm), and radioactivity was quantitated by fraction collection.

The results of the ACN:water and potassium phosphate buffer extractions were comparable in terms of the amount of radioactivity released and the composition of the extractable residues as determined by TLC and HPLC. Although TLC and HPLC analysis of the potassium phosphate buffer solutions of beans and forage, and the amylase hydrolysate of S-methyl-treated forage identified acephate and related metabolites, similar analyses of the remaining hydrolysates merely separated components into polar, medium polar, and nonpolar products. The registrant stated that the common distribution of radioactivity in polar and nonpolar regions following reverse-phase HPLC of lignin, hemicellulose, and cellulose fractions of beans and forage suggested that S-methyl- and carbonyl-labelled moieties had been metabolized to $^{14}CO_2$ and incorporated into natural plant components via photosynthesis.

The registrant conducted the following additional analyses to further characterize\identify the radioactive residues in the ACN:water extracts: (i) extraction and re-chromatography via TLC of isolated radiolabelled zones from the initial TLC analyses; (ii) strong anion exchange solid-phase extraction (SPE) to compare polar metabolites from the S-methyl- and carbonyl-treated bean forage and from the lettuce metabolism study discussed below; (iii) anion exchange chromatography followed by normal-phase TLC; (iv) C-18 SPE followed by reverse-phase HPLC; and (v) derivatization of anionic and polar metabolites. These procedures essentially confirmed the findings of the initial TLC and HPLC analyses.

Additional characterization of unknowns P1, O', and P2 was attempted by TLC following C-18 and anion exchange SPE purification of the polar fraction obtained for NMR analyses. The analyses indicated that the three unknowns are very polar non-anionic constituents.

The distribution and characterization/identification of ^{14}C-activity in the extracts of beans and forage are presented in Tables 2 and 3. A summary of the characterized and identified ^{14}C-residues is presented in Tables 4 and 5.

Table 2. Distribution and characterization/identification of radioactive residues in beans treated with [S-methyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	% TRR	ppm	Characterization/Identification [a]
Whole beans (TRR = 16.216 ppm) - first subsample			
ACN:water	45.76	7.420	TLC analysis resolved: Acephate 13.5% TRR 2.196 ppm Methamidophos 7.3% TRR 1.187 ppm SMPT 8.6% TRR 1.388 ppm Unknown P1 6.9% TRR 1.120 ppm Unknown O' 3.2% TRR 0.527 ppm Unknown P2 5.4% TRR 0.883 ppm
ACN:0.1 N HCl	1.21	0.196	TLC analysis resolved: Acephate 0.2% TRR 0.040 ppm Origin 0.8% TRR 0.132 ppm
6 N HCl	29.67	4.811	HPLC analysis indicated that the majority of the TRR were comprised of polar products, with some regions of less polar and nonpolar products.
Unextracted	4.83	0.783	Not further analyzed (N/A).
Whole beans (TRR = 16.216 ppm) - second subsample			
Potassium phosphate buffer	64.06	10.388	TLC analysis of the phosphate buffer extract tentatively identified acephate, methamidophos, SMPT, and metabolite P1; HPLC analysis confirmed the presence of acephate and methamidophos. Amylase hydrolysate 6.3% TRR 1.028 ppm E-Pronase hydrolysate 2.0% TRR 0.326 ppm Pectin hydrolysate 1.9% TRR 0.305 ppm Lignin hydrolysate 1.2% TRR 0.190 ppm Hemicellulose hydrolysate 7.5% TRR 1.221 ppm Cellulose hydrolysate 1.2% TRR 0.193 ppm
Unextracted	1.91	0.310	N/A.
Bean forage (TRR = 74.433 ppm) - first subsample			
ACN:water	101.96	75.893	TLC analysis resolved: Acephate 74.1% TRR 55.174 ppm Methamidophos 7.6% TRR 5.692 ppm SMPT 14.2% TRR 10.549 ppm Unknown P1 2.7% TRR 1.973 ppm Unknown O' 1.5% TRR 1.138 ppm Unknown P2 0.9% TRR 0.683 ppm
ACN:0.1 N HCl	0.90	0.670	TLC analysis resolved: Acephate 0.3% TRR 0.203 ppm Origin 0.4% TRR 0.267 ppm
6 N HCl	4.23	3.149	HPLC analysis indicated that the majority of the TRR were comprised of polar products, with some regions of less polar and nonpolar products.
Unextracted	2.75	2.047	N/A.

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification [a]
Bean forage (TRR = 74.433 ppm) - second subsample			
Potassium phosphate buffer	97.13	72.296	TLC analysis of the phosphate buffer extract tentatively identified acephate, methamidophos, SMPT, and metabolite P1; HPLC analysis confirmed the presence of acephate and methamidophos. Amylase hydrolysate 3.1% TRR 2.337 ppm E-Pronase hydrolysate 1.0% TRR 0.752 ppm Pectin hydrolysate1.0% TRR 0.752 ppm Lignin hydrolysate 0.9% TRR 0.662 ppm Hemicellulose hydrolysate 2.0% TRR 1.474 ppm Cellulose hydrolysate 0.2% TRR 0.171 ppm HPLC analysis of amylase hydrolysates identified substantial levels of acephate and some methamidophos.
Unextracted	0.42	0.313	N/A.

[a] TLC identification of acephate and methamidophos was confirmed by GC/MS. TLC identification of SMPT and OMAPAA was confirmed by ^{31}P-NMR.

(continued; footnotes follow)

Table 3. Distribution and characterization/identification of radioactive residues in beans treated with [carbonyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	% TRR	ppm	Characterization/Identification [a]
Whole beans (TRR = 12.385 ppm) - first subsample			
ACN:water	82.54	10.223	TLC analysis resolved: Acephate 14.7% TRR 1.820 ppm SMPT 7.0% TRR 0.869 ppm OMAPAA 56.6% TRR 7.013 ppm Origin 2.4% TRR 0.296 ppm
ACN:0.1 N HCl	1.10	0.136	TLC analysis resolved: Acephate 0.1% TRR 0.014 ppm Origin 0.8% TRR 0.099 ppm
6 N HCl	8.56	1.060	HPLC analysis indicated that the majority of the TRR were comprised of polar products, with some regions of less polar and nonpolar products.
Unextracted	3.51	0.435	N/A.
Whole beans (TRR = 12.385 ppm) - second subsample			
Potassium phosphate buffer	86.33	10.692	TLC analysis of the phosphate buffer extract tentatively identified acephate, SMPT, and OMAPAA; HPLC analysis confirmed the presence of acephate and polar metabolites. Amylase hydrolysate 2.8% TRR 0.347 ppm E-Pronase hydrolysate 1.0% TRR 0.121 ppm Pectin hydrolysate 0.9% TRR 0.116 ppm Lignin hydrolysate 0.4% TRR 0.050 ppm Hemicellulose hydrolysate 2.8% TRR 0.349 ppm Cellulose hydrolysate 0.3% TRR 0.042 ppm
Nonextractable	0.66	0.082	N/A.
Bean forage (TRR = 85.278 ppm) - first subsample			
ACN:water	95.66	81.577	TLC analysis resolved: Acephate 62.1% TRR 52.943 ppm SMPT 6.5% TRR 5.547 ppm OMAPAA 22.5% TRR 19.171 ppm Origin 2.4% TRR 2.039 ppm Unknown A' 2.2% TRR 1.876 ppm
ACN:0.1 N HCl	1.17	0.998	TLC analysis resolved: Acephate 0.4% TRR 0.370 ppm Origin 0.6% TRR 0.521 ppm
6 N HCl	4.27	3.641	HPLC analysis indicated that the majority of the TRR was comprised of polar products, with some regions of less polar and nonpolar products.
Unextracted	1.84	1.569	N/A.
Bean forage (TRR = 85.278 ppm) - second subsample			
Potassium phosphate buffer	93.21	79.487	TLC analysis of the phosphate buffer extract tentatively

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification [a]
			identified acephate, methamidophos, SMPT, and metabolite P1; HPLC analysis confirmed the presence of acephate and methamidophos. Amylase hydrolysate 1.4% TRR 1.202 ppm E-Pronase hydrolysate 0.3% TRR 0.281 ppm Pectin hydrolysate0.6% TRR 0.486 ppm Lignin hydrolysate 0.5% TRR 0.392 ppm Hemicellulose hydrolysate 1.0% TRR 0.878 ppm Cellulose hydrolysate 0.1% TRR 0.060 ppm
Nonextractable	0.18	0.154	N/A.

[a] TLC identification of acephate and methamidophos was confirmed by GC/MS. TLC identification of SMPT and OMAPAA was confirmed by ^{31}P-NMR.

(continued; footnotes follow)

Table 4. Summary of characterized/identified ^{14}C-residues in beans treated with [S-methyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	Beans (TRR = 16.216)		Forage (TRR = 74.433)	
	% TRR	ppm	% TRR	ppm
Identified [a]				
Acephate	13.7	2.236	74.4	55.377
Methamidophos	7.3	1.187	7.6	5.692
SMPT	8.6	1.388	14.2	10.549
Total identified	29.6	4.811	96.2	71.618
Characterized				
Unknown P1	6.9	1.120	2.7	1.973
Unknown O'	3.2	0.527	1.5	1.138
Unknown P2	5.4	0.883	0.9	0.683
Origin	0.8	0.132	0.4	0.267
Amylase hydrolysate	6.3	1.028	3.1	2.337
E-Pronase hydrolysate	2.0	0.326	1.0	0.752
Pectin hydrolysate	1.9	0.305	1.0	0.752
Lignin hydrolysate	1.2	0.190	0.9	0.662
Hemicellulose hydrolysate	7.5	1.221	2.0	1.474
Cellulose hydrolysate	1.2	0.193	0.2	0.171
Total identified/characterized	66.0	10.736	109.9	81.827
Nonextractable	1.9	0.310	0.4	0.313

[a] See Figure 1 for the chemical structures of identified metabolites.

Table 5. Summary of characterized/identified ^{14}C-residues in beans treated with [carbonyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	Beans (TRR = 12.385)		Forage (TRR = 85.278)	
	% TRR	ppm	% TRR	ppm
Identified [a]				
Acephate	14.8	1.834	62.5	53.313
SMPT	7.0	0.869	6.5	5.547
OMAPAA	56.6	7.013	22.5	19.171
Total identified	78.4	9.716	91.5	78.031
Characterized				
Unknown A'	--	--	2.2	1.876
Origin	3.2	0.395	3.0	2.560
Amylase hydrolysate	2.8	0.347	1.4	1.202
E-Pronase hydrolysate	1.0	0.121	0.3	0.281
Pectin hydrolysate	0.9	0.116	0.6	0.486
Lignin hydrolysate	0.4	0.050	0.5	0.392
Hemicellulose hydrolysate	2.8	0.349	1.0	0.878
Cellulose hydrolysate	0.3	0.042	0.1	0.060
Total identified/characterized	89.8	11.136	100.6	85.766
Nonextractable	0.7	0.082	0.2	0.154

[a] See Figure 1 for the chemical structures of identified metabolites.

Figure 1. Acephate and its metabolites in beans (MRID 43971603), cotton (MRID 44037801), lettuce (MRID 43971602), lactating goats (MRID 43971604) and laying hens (MRID 44037803).

Common Name Chemical Name	Structure	Substrate
Acephate **O,S-dimethyl acetylphosphoramidothioate**		**Beans and bean forage** **Cotton seed meal, seed hulls, and gin trash** **Lettuce** **Goat milk, kidney, liver, and muscle** **Poultry egg white, egg yolk, fat, muscle, and liver**
Methamidophos **O,S-dimethyl phosphoramidothioate**		**Beans and bean forage** **Cotton seed meal and gin trash** **Lettuce** **Goat milk, kidney, liver, and muscle** **Poultry egg white, fat, and muscle**
SMPT **S-methyl N-acetylphosphoramidothioate**		**Beans and bean forage** **Cotton seed meal, seed hulls, and gin trash** **Lettuce** **Goat milk, kidney, and liver** **Poultry egg white, egg yolk, fat, muscle, and liver**
SMPAA **S-methyl phosphoramidothioate**		**Cotton seed meal, seed hulls, and gin trash** **Goat kidney and liver** **Poultry egg white, egg yolk, fat, and liver**
DMPT **O,S-dimethyl phosphorothioate**		**Cotton gin trash** **Poultry egg white, egg yolk, fat, and liver**
OMAPAA **O-methyl N-acetylphosphoramidate**		**Beans and bean forage** **Cotton seed meal, seed hulls, and gin trash** **Lettuce** **Goat kidney and liver** **Poultry egg yolk, fat, muscle, and liver**
Acetamide		**Cotton seed meal, seed hulls, and gin trash**

(continued)

Common Name Chemical Name	Structure	Substrate
		Goat milk and liver **Poultry egg white, egg yolk, fat, muscle, and liver**

Storage stability

All RAC samples, homogenates, and fractions were stored frozen (<0 C) prior to analysis. Analysis of bean and forage extracts was initiated within 33 days of harvest. Although dates of final sample analyses were not provided, an HPLC chromatogram included in the submission indicates that analyses were being conducted up to 414 days (~14 months) after harvest. The registrant submitted storage stability data demonstrating that the metabolite profiles of beans and forage extracted 19 days after harvest and analyzed 78 days after harvest were similar to those of beans and forage extracted and analyzed ~16 months after harvest. The registrant must supply the dates of sample analyses to confirm the adequacy of the storage stability data.

Study summary

The bean metabolism study is acceptable, pending submission of the outstanding storage stability information. Total radioactive residues were 16.216 ppm or 12.385 ppm in whole beans and 74.433 ppm or 85.278 ppm in bean forage collected 14 days following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate, respectively, at 1 lb ai/A/application (1x the maximum registered single application rate).

In beans from the S-methyl treatment, ~66 of TRR in whole beans and ~110 of TRR in forage was characterized/identified. Acephate was identified at 13.7 of TRR (2.236 ppm) in whole beans and 74.4 of TRR (55.377 ppm) in bean forage. The metabolites methamidophos and SMPT were identified at 7.3 of TRR (1.187 ppm) and 8.6 of TRR (1.388 ppm), respectively, in whole beans, and at 7.6 of TRR (5.692 ppm) and 14.2 of TRR (10.549 ppm), respectively, in forage.

In beans from the carbonyl treatment, ~90 of TRR in whole beans and ~101 of TRR in forage was characterized/identified. Acephate was identified at 14.8 of TRR (1.834 ppm) in whole beans and 62.5 of TRR (53.313 ppm) in bean forage. The metabolites OMAPAA and SMPT were identified at 56.6 of TRR (7.013 ppm) and 7.0 of TRR (0.869 ppm), respectively, in whole beans, and at 22.5 of TRR (19.171 ppm) and 6.5 of TRR (5.547 ppm), respectively, in forage.

(continued)

Cotton

Use patterns registered to Valent

The 75 SC (EPA Reg. Nos. 59639-26 and 59639-89) and 90 SC (EPA Reg. Nos. 59639-33 and 59639-86) formulations are registered by Valent for multiple foliar applications to cotton at 0.14-1.0 lb ai/A/application using ground or aerial equipment. Applications are to be made in a minimum of 10 gal/A of water when using ground equipment, and a minimum of 1-3 gal/A of water (5 gal/A in CA) when using aerial equipment. Applications may be made alone or as a tank mix with other insecticides. The established PHI is 21 days. No maximum seasonal rate or maximum number of applications has been established. The feeding of gin trash to livestock or grazing of animals on treated areas is prohibited. *NOTE: CBRS has determined that this restriction is not enforceable. This restriction should be deleted from the labels and residue data submitted for cotton gin byproducts.*

The 75 SC (EPA Reg. No. 59639-26) and 90 SC (EPA Reg. No. 59639-33) formulations are registered by Valent for application as a dry powder to cottonseed in hopper/planter boxes at 0.14-0.19 lb ai/A. Use is limited to all states except AZ, CA, and the Blacklands of TX. The use of treated seed for food/feed purposes or for processing for oil is prohibited.

The 80 SC formulations (EPA Reg. Nos. 59639-29 and 59639-85) are registered by Valent for application as a slurry seed treatment at 0.4 lb ai/100 lbs of cottonseed. The use of treated seed for food/feed purposes or for processing for oil is prohibited.

NOTE: Restrictions for the use of treated seed for food/feed purposes or for processing for oil are not considered to be practical or enforceable. These restrictions should be deleted from the labels and additional residue data submitted.

The 75 SC (EPA Reg. Nos. 59639-26 and 59639-89) and 90 SC (EPA Reg. Nos. 59639-33 and 59639-86) formulations are also registered for application as a single in-furrow spray at planting to cotton at 0.5-1.0 lb ai/A in a minimum of 3 gal/A of water using ground equipment.

The 15 G formulation (EPA Reg. No. 59639-75) is registered by Valent for an in-furrow application to cotton at planting at 0.75-1.0 lb ai/A using ground equipment.

In-life phase

Valent submitted data (1996; MRID 44037801) pertaining to the metabolism of [S-methyl-^{14}C]acephate and [carbonyl-^{14}C]acephate in cotton. The in-life and analytical phases of the study

(continued)

were conducted at ABC-CA Laboratories (Madera, CA); ^{31}P-NMR analyses were conducted at Acorn NMR, Inc. (Fremont, CA). The test substances, [S-methyl-^{14}C]acephate (specific activity 52.1 mCi/mmol) and [carbonyl-^{14}C]acephate (specific activity 37.7 mCi/mmol), were isotopically diluted with unlabelled acephate and diluted with ACN prior to application to give final specific activities of 2.17 mCi/mmol and 2.16 mCi/mmol, respectively; the radiochemical purity for both test substances was >95 . Cotton in each of two treatment plots received three foliar applications by hand trigger sprayer of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate at ~1 lb ai/A/application (1x the maximum single application rate) for a total application rate of ~3 lb ai/A. Cotton plants in a third plot served as positive controls and received unlabeled acephate at 2.0 lb ai/A/application, and a fourth plot was established for untreated control plants. Applications were made at 7-day retreatment intervals; mature cotton plants were harvested 21 days after final application. Adequate information pertaining to plant growth, maintenance, weather conditions, test material and application, and harvest was provided.

Samples of open cotton bolls, unopened bolls, dry bracts, and foliage were harvested separately from each plot; the dry bract samples were considered to represent gin trash for the purposes of this study. Samples were placed in plastic bags and were stored immediately in field freezers. Open cotton boll samples in each plot were ginned to separate seeds from lint; no further processing or analysis was conducted on lint or unopened bolls. All samples were stored frozen (<0 C) at ABC-CA until processing.

Total radioactive residues (TRR)

Samples of cotton seed were initially rinsed with methanol, then air-dried at room temperature, and homogenized with dry ice. Seed samples were subsequently subjected to micro-milling, and were sieved to separate the fuzzy seed hull from seed meal. Gin trash and foliage samples were homogenized with dry ice. Triplicate aliquots of homogenized plant tissues were combusted and radioassayed by LSC; the methanol rinse from cotton seed was concentrated and assayed directly by LSC. The LOD for combustion/LSC was 0.003 ppm for samples from both treatments. The TRR in treated cotton matrices are presented in Table 6.

Table 6. Total radioactive residues (TRR) in cotton matrices following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate at 1.0 lb ai/A/application.

	TRR, ppm [^{14}C]acephate equivalents	
Matrix	**S-Methyl**	**Carbonyl**
Seed meal	**3.100**	**0.511**
Seed hulls	**2.043**	**0.462**
Gin trash (bracts)	**12.756**	**13.225**

(continued)

Foliage	**62.260**	**35.818**

^{14}C-Residues in both sets of control plants were below the LOD; residues in the methanol rinse were 0.001 ppm as determined by LSC. No further analysis was conducted on the methanol rinse or on cotton foliage; cotton foliage is not considered to be a significant livestock feed item (Table 1 of OPPTS 860.1000).

Extraction and hydrolysis of residues

Homogenized samples of cotton seed, hulls, and gin trash were subjected to extraction and hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction procedures are summarized below.

Cotton samples from each treatment were extracted first with hexane:ACN (1:1, v:v) and then with ACN; after each extraction the mixtures were filtered, and the resulting filtrates were combined and transferred to a separatory funnel. Following phase separation, the ACN fraction was partitioned twice with hexane, and the nonextractable residues remaining from seed meal and hulls were re-extracted with hexane. All hexane fractions were pooled (Hexane-A), and the nonextractable residues were extracted twice with water. The ACN, Hexane-A, and aqueous fractions of cotton matrices were reserved for TLC, and/or preparative TLC, HPLC, and ^{31}P-NMR analysis.

To generate additional hexane-soluble residues for lipid analysis, a subsample of S-methyl-treated seed meal was extracted four times with hexane. The resulting hexane extracts (Hexane-B) were pooled and partitioned with ACN, and reserved for further analysis. Additional subsamples of gin trash were subjected to the same extraction scheme on a larger scale for purification and identification of metabolites.

Nonextractable residues were subjected to the following sequential hydrolysis steps: protein digestion with pronase (room temperature, for 24 hours); acid hydrolysis with 1 M HCl (reflux for 2 hours); and base hydrolysis with 20 aqueous NaOH (reflux for 2 hours). Following base hydrolysis, the mixture was filtered to separate insoluble cellulose from the base-soluble hydrolysate. The pronase hydrolysate was lyophilized and analyzed by TLC. The final filtrate was adjusted to pH 1.0 with concentrated HCl and centrifuged to precipitate lignins from the acid-soluble hydrolysate.

The distribution of ^{14}C-activity in the extracts and hydrolysates of cotton matrices is presented in Tables 7 and 8.

(continued)

Characterization/identification of residues

Extracts and hydrolysates were analyzed by TLC and HPLC. Metabolites were identified by cochromatography with the following reference standards: acephate, methamidophos, SMPT, DMPT, SMPAA, OMAPAA, and acetamide. TLC identifications of acephate and metabolites were confirmed by HPLC and ^{31}P-NMR for acephate and methamidophos, ^{31}P-NMR for SMPT, DMPT, and OMAPAA, and HPLC for acetamide; SMPAA was identified by TLC only.

TLC analyses were conducted on precoated silica gel (F254) plates; radioactivity was visualized using a radioscanner, and quantitated by imaging software. Nonlabeled components were visualized by exposure to iodine vapor. For the majority of TLC analyses, plates were developed in the following solvent systems: ammonium hydroxide:benzene:isopropanol (3:2:16, v:v:v; System I); petroleum ether:diethyl ether:acetic acid (80:20:1, v:v:v; System II); or isopropyl ether:acetic acid (24:1, v:v), followed by petroleum ether:diethyl ether:acetic acid (90:10:1, v:v:v; System III). Solvent System III was designed to separate triglycerides in hexane extracts from diglycerides, monoglycerides, phospholipids, and hydrocarbons.

HPLC analysis was conducted using a Phenomenex Ultremex 5 C-18 column; radioactivity was quantitated by a radioflow detector and by fraction collection. Analysis of metabolites in various extracts (Method I) involved a gradient mobile phase of 0.1 trifluoroacetic acid in water and 0.1 trifluoroacetic acid in ACN; nonlabeled standards were detected by UV (230 nm). The registrant reported that this method successfully isolated acephate and methamidophos. Analysis of free and derivatized fatty acids in hexane extracts (Method II) involved a gradient mobile phase of acidified ACN:tetrahydrofuran:water (2:1:1, v:v:v) and acidified ACN:tetrahydrofuran (1:1, v:v); nonlabeled standards were detected by UV (215 nm).

The Hexane-A and Hexane-B fractions from seed meal were subjected to the following additional analyses to characterize/identify triglycerides and fatty acids: (i) partitioning with 5 aqueous sodium bicarbonate to separate triglycerides (hexane phase) from free fatty acids (aqueous phase); and (ii) saponification via 2 M potassium hydroxide in ethanol:water (1:1, v:v) followed by partitioning with petroleum ether and derivatization of isolated fatty acids to p-bromophenacyl esters. These procedures confirmed the presence of [^{14}C]triglycerides in the hexane-soluble residues; following saponification and derivatization, peaks corresponding to oleic/palmitic and linoleic acid were identified by HPLC.

The aqueous extract of gin trash from each treatment was subjected to additional purification involving lyophilization and solid-phase extraction on a C-18 column to facilitate ^{31}P-NMR analysis of metabolites. The combined aqueous extract of S-methyl-treated seed meal and hull

(continued)

were also subjected ^{31}P-NMR analysis which confirmed that the highly polar material remaining at the origin did not contain any organophosphate metabolites with the S-methyl group.

The distribution and characterization/identification of ^{14}C-activity in the extracts of cotton and forage are presented in Tables 7 and 8. A summary of the characterized and identified ^{14}C-residues is presented in Tables 9 and 10.

(continued)

Table 7. Distribution and characterization/identification of radioactive residues in cotton matrices treated with [S-methyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Seed meal (TRR = 3.100 ppm)			
Hexane-A	35.2	1.091	Lipids in this fraction were characterized as triglycerides. TLC analysis resolved a nonpolar, diffuse, but homogeneous spot corresponding to the triglyceride tributyrin. Results were confirmed by solvent partitioning with aqueous sodium bicarbonate and by saponification of the hexane fraction.
ACN	2.8	0.086	TLC analysis resolved: Acephate 0.8% TRR 0.025 ppm Methamidophos 0.5% TRR 0.014 ppm SMPT 0.5% TRR 0.017 ppm SMPAA 0.9% TRR 0.029 ppm
Aqueous	18.5	0.575	TLC analysis resolved: SMPT 2.5% TRR 0.079 ppm Origin 16.0% TRR 0.496 ppm
Nonextractable	38.5	1.193	Pronase hydrolysate 2.3% TRR 0.071 ppm Acid hydrolysate 14.4% TRR 0.445 ppm Lignin fraction 2.7% TRR 0.085 ppm Base hydrolysate 5.0% TRR 0.156 ppm Cellulose fraction 8.3% TRR 0.256 ppm
Seed hulls (TRR = 2.043 ppm)			
Hexane-A	12.5	0.256	Lipids in this fraction were characterized as triglycerides; additional work-up of seed meal extracts confirmed this conclusion.
ACN	3.9	0.080	TLC analysis resolved: Acephate 2.1% TRR 0.043 ppm SMPT 1.1% TRR 0.022 ppm SMPAA 0.7% TRR 0.015 ppm
Aqueous	17.2	0.351	TLC analysis resolved: SMPT 2.8% TRR 0.057 ppm Origin 14.4% TRR 0.294 ppm
Nonextractable	59.3	1.212	Pronase hydrolysate 3.3% TRR 0.068 ppm Acid hydrolysate 7.4% TRR 0.151 ppm Lignin fraction 10.6% TRR 0.216 ppm Base hydrolysate 12.4% TRR 0.253 ppm Cellulose fraction 17.4% TRR 0.356 ppm
Nonextractable	N/R	N/R	N/A.
Gin trash (TRR = 12.756 ppm)			
Hexane-A	0.4	0.046	Lipids in this fraction were characterized as triglycerides; additional work-up of seed meal extracts confirmed this conclusion.

(continued; footnotes follow)

Fraction	% TRR [a]	ppm	Characterization/Identification [b]
ACN	42.3	5.397	TLC analysis resolved: Acephate 36.0% TRR 4.598 ppm Methamidophos 1.5% TRR 0.189 ppm SMPT 3.2% TRR 0.410 ppm SMPAA 0.8% TRR 0.108 ppm DMPT 0.7% TRR 0.086 ppm
Aqueous	36.3	4.631	TLC analysis resolved: Acephate 1.7% TRR 0.222 ppm SMPT 23.2% TRR 2.964 ppm DMPT 3.3% TRR 0.417 ppm Origin 8.1% TRR 1.033 ppm
Nonextractable	12.9	1.650	Pronase hydrolysate 3.2% TRR 0.403 ppm Acid hydrolysate 2.0% TRR 0.255 ppm Lignin fraction 1.7% TRR 0.211 ppm Base hydrolysate 2.8% TRR 0.352 ppm Cellulose fraction 0.7% TRR 0.089 ppm TLC analysis of the pronase hydrolysate indicated that SMPT was released.
Unextracted	N/R	N/R	N/A.

[a] Calculated by the study reviewer; reported % TRR had been normalized to 100% by the registrant.
[b] TLC identifications of acephate and metabolites were confirmed by HPLC and ^{31}P-NMR for acephate and methamidophos, ^{31}P-NMR for SMPT and DMPT; SMPAA was identified by TLC only.
[c] N/R = not reported.

(continued; footnotes follow)

Table 8. Distribution and characterization/identification of radioactive residues in cotton matrices treated with [carbonyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Seed meal (TRR = 0.511 ppm)			
Hexane-A	20.0	0.102	Lipids in this fraction were characterized as triglycerides. TLC analysis resolved a nonpolar, diffuse, but homogeneous spot corresponding to the triglyceride tributyrin. Results were confirmed by solvent partitioning with aqueous sodium bicarbonate and by saponification of the hexane fraction.
ACN	7.0	0.036	TLC analysis resolved: Acephate 2.0% TRR 0.010 ppm SMPT 1.0% TRR 0.005 ppm OMAPAA 1.4% TRR 0.007 ppm Acetamide 2.5% TRR 0.013 ppm
Aqueous	37.4	0.191	TLC analysis resolved: OMAPAA 20.4% TRR 0.104 ppm Origin 17.0% TRR 0.087 ppm
Nonextractable	36.4	0.186	Pronase hydrolysate 5.5% TRR 0.028 ppm Acid hydrolysate 11.9% TRR 0.061 ppm Lignin fraction 0.8% TRR 0.004 ppm Base hydrolysate 2.9% TRR 0.015 ppm Cellulose fraction 6.5% TRR 0.033 ppm
Unextracted	N/R [c]	N/R	N/A.
Seed hulls (TRR = 0.462 ppm)			
Hexane-A	3.7	0.017	Lipids in this fraction were characterized as triglycerides; additional work-up of seed meal extracts confirmed this conclusion.
ACN	22.9	0.106	TLC analysis resolved: Acephate 7.1% TRR 0.033 ppm SMPT 2.4% TRR 0.011 ppm OMAPAA 4.1% TRR 0.019 ppm Acetamide 9.3% TRR 0.043 ppm
Aqueous	31.6	0.146	TLC analysis resolved: OMAPAA 19.5% TRR 0.090 ppm Origin 12.1% TRR 0.056 ppm
Nonextractable	39.2	0.181	Pronase hydrolysate 6.5% TRR 0.030 ppm Acid hydrolysate 9.5% TRR 0.044 ppm Lignin fraction 4.1% TRR 0.019 ppm Base hydrolysate 8.7% TRR 0.040 ppm Cellulose fraction 6.7% TRR 0.031 ppm
Unextracted	N/R	N/R	N/A.
Gin trash (TRR = 13.225 ppm)			
Hexane-A	1.3	0.166	Lipids in this fraction were characterized as

(continued; footnotes follow)

Fraction	% TRR [a]	ppm	Characterization/Identification [b]
			triglycerides; additional work-up of seed meal extracts confirmed this conclusion.
ACN	38.7	5.113	TLC analysis resolved: Acephate 34.4% TRR 4.551 ppm SMPT 1.8% TRR 0.235 ppm OMAPAA 0.9% TRR 0.118 ppm Acetamide 1.6% TRR 0.205 ppm
Aqueous	47.4	6.275	TLC analysis resolved: Acephate 2.9% TRR 0.377 ppm SMPT 14.2% TRR 1.883 ppm OMAPAA 24.0% TRR 3.175 ppm Origin 6.4% TRR 0.841 ppm
Nonextractable	6.0	0.787	Pronase hydrolysate 1.9% TRR 0.248 ppm Acid hydrolysate 1.8% TRR 0.240 ppm Lignin fraction 0.2% TRR 0.023 ppm Base hydrolysate 0.4% TRR 0.058 ppm Cellulose fraction 0.5% TRR 0.067 ppm
Unextracted	N/R	N/R	N/A.

[a] Calculated by the study reviewer; reported % TRR had been normalized to 100% by the registrant.
[b] TLC identifications of acephate and metabolites were confirmed by HPLC and ^{31}P-NMR for acephate ^{31}P-NMR for OMAPAA, and HPLC for acetamide.
[c] N/R = Not reported.

(continued; footnotes follow)

Table 9. Summary of characterized/identified ^{14}C-residues in cotton matrices treated with [S-methyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	Seed Meal (TRR = 3.100 ppm)		Seed Hulls (TRR = 2.043 ppm)		Gin Trash (TRR = 12.756 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]						
Acephate	0.8	0.025	2.1	0.043	37.7	4.821
Methamidophos	0.5	0.014	--	--	1.5	0.189
SMPT	3.0	0.096	3.9	0.079	26.4	3.374
SMPAA	0.9	0.029	0.7	0.015	0.8	0.108
DMPT	--	--	--	--	4.0	0.503
Total identified	5.2	0.164	6.7	0.137	70.4	8.995
Characterized						
Lipids/triglycerides	35.2	1.091	12.5	0.256	0.4	0.046
Origin	16.0	0.496	14.4	0.294	8.1	1.033
Pronase hydrolysate	2.3	0.071	3.3	0.068	3.2	0.403
Acid hydrolysate	14.4	0.445	7.4	0.151	2.0	0.255
Lignin fraction	2.7	0.085	10.6	0.216	1.7	0.211
Base hydrolysate	5.0	0.156	12.4	0.253	2.8	0.352
Cellulose fraction	8.3	0.256	17.4	0.356	0.7	0.089
Total identified/characterized	89.1	2.764	84.7	1.731	89.3	11.384
Unextracted	N/R [b]	N/R	N/R	N/R	N/R	N/R

[a] See Figure 1 for the chemical structures of identified metabolites.
[b] N/R = Not reported.

Table 10. Summary of characterized/identified ^{14}C-residues in cotton treated with [carbonyl-^{14}C]acephate at ~3 lb ai/A.

Fraction	Seed Meal (TRR = 0.511 ppm)		Seed Hulls (TRR = 0.462 ppm)		Gin Trash (TRR = 13.225 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]						
Acephate	2.0	0.010	7.1	0.033	37.3	4.928
SMPT	1.0	0.005	2.4	0.011	16.0	2.118
OMAPAA	21.8	0.111	23.6	0.109	24.9	3.293
Acetamide	2.5	0.013	9.3	0.043	1.6	0.205
Total identified	27.3	0.140	42.4	0.196	79.8	10.544
Characterized						
Lipids/triglycerides	20.0	0.102	3.7	0.017	1.3	0.166
Origin	17.0	0.087	12.1	0.056	6.4	0.841
Pronase hydrolysate	5.5	0.028	6.5	0.030	1.9	0.248
Acid hydrolysate	11.9	0.061	9.5	0.044	1.8	0.240
Lignin fraction	0.8	0.004	4.1	0.019	0.2	0.023
Base hydrolysate	2.9	0.015	8.7	0.040	0.4	0.058
Cellulose fraction	6.5	0.033	6.7	0.031	0.5	0.067
Total identified/characterized	91.9	0.470	93.7	0.433	92.3	12.187
Unextracted	N/R [b]	N/R	N/R	N/R	N/R	N/R

[a] See Figure 1 for the chemical structures of identified metabolites.
[b] N/R = Not reported.

Storage stability

All RAC samples, homogenates, and fractions were stored frozen (<0 C) prior to analysis. Dates of sample analysis were not included in the submission; however, a ^{31}P-NMR spectrum included with the submission indicates that analyses were being conducted up to 384 days (~13 months) after harvest. The registrant stated that the metabolite profiles of cotton seed meal and hull did not change following 2 months of frozen storage; no supporting data were submitted. These data do not adequately support the estimated storage intervals for samples used in the cotton metabolism study. The registrant must supply the dates of analysis of samples, and provide data indicating that the metabolite profile in cotton commodities did not change over the intervals for which samples were stored.

Study summary

The cotton metabolism study is acceptable, pending submission of the outstanding storage stability data. Total radioactive residues were 3.100 ppm or 0.511 ppm in cotton seed meal, 2.043 ppm or 0.462 ppm in cotton seed hulls, and 12.756 ppm or 13.225 ppm in gin trash collected 21 days following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate, respectively, at 1 lb ai/A/application (1x the maximum single application rate). The registrant attributed the higher TRR in S-methyl-treated seed meal and hulls more rapid cleavage of the S-methyl group, which resulted in more rapid incorporation into plant parts.

In cotton commodities from the S-methyl treatment, ~85-89 of TRR was characterized/identified. Acephate was identified at 0.8 of TRR (0.025 ppm) in seed meal, 2.1 of TRR (0.043 ppm) in seed hulls, and 37.7 of TRR (4.821 ppm) in gin trash. Methamidophos was identified at 0.5 of TRR (0.014 ppm) and 1.5 of TRR (0.189 ppm), respectively, in seed meal and gin trash. The metabolites SMPT and SMPAA were identified at 3.0 of TRR (0.096 ppm) and 0.9 of TRR (0.029 ppm), respectively, in seed meal, at 3.9 of TRR (0.079 ppm) and 0.7 of TRR (0.015 ppm), respectively, in seed hulls, and at 26.4 of TRR (3.374 ppm) and 0.8 of TRR (0.108 ppm), respectively, in gin trash. The metabolite DMPT was identified in gin trash only at 4.0 of TRR (0.503 ppm). In addition, a lipid component characterized as triglycerides comprised 35.2 of TRR (1.091 ppm) in seed meal and 12.5 of TRR (0.256 ppm) in seed hulls.

In cotton commodities from the carbonyl treatment, ~92-94 of TRR was characterized/identified. Acephate was identified at 2.0 of TRR (0.010 ppm) in seed meal, 7.1 of TRR (0.033 ppm) in seed hulls, and 37.3 of TRR (4.928 ppm) in gin trash. The metabolites SMPT, OMAPAA, and acetamide were identified at 1.0 of TRR (0.005 ppm), 21.8 of TRR (0.111 ppm), and 2.5 of TRR (0.013 ppm), respectively, in seed meal, at 2.4 of TRR (0.011 ppm), 23.6 of TRR (0.109 ppm), and 9.3 of TRR (0.043 ppm), respectively, in seed

hulls, and at 16.0 of TRR (2.118 ppm), 24.9 of TRR (3.293 ppm), and 1.6 of TRR (0.205 ppm), respectively, in gin trash. In addition, a lipid component characterized as triglycerides comprised 20.0 of TRR (0.102 ppm) in seed meal and 3.7 of TRR (0.017 ppm) in seed hulls.

Lettuce

Use patterns registered to Valent

The 75 SC formulations (EPA Reg. Nos. 59639-26 and 59639-89) are registered by Valent for multiple foliar applications to head lettuce (crisphead type only) at 0.5-1.0 lb ai/A/application using ground or aerial equipment. Applications should be made in a minimum of 10 gal/A of water when using ground equipment, and 5 gal/A of water when using aerial equipment. The established PHI is 21 days and the maximum seasonal rate is 5.0 lb ai/A. The feeding of trimmings to livestock or the grazing of animals on treated areas is prohibited.

In-life phase

Valent submitted data (1996; MRID 43971602) pertaining to the metabolism of [S-methyl-^{14}C]acephate and [carbonyl-^{14}C]acephate in lettuce. The in-life phase of the study was conducted at Plant Sciences, Inc. (Watsonville, CA). The test substances, [S-methyl-^{14}C]acephate (specific activity 52.1 mCi/mmol) and [carbonyl-^{14}C]acephate (specific activity 37.7 mCi/mmol), were isotopically diluted with unlabelled acephate and diluted with deionized water prior to application to give final specific activities of 3.41 mCi/mmol and 3.56 mCi/mmol, respectively; the radiochemical purity for both test substances was >96 . Lettuce in each of two treatment boxes received three foliar applications by hand trigger sprayer of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate at ~1 lb ai/A/application for a total application rate of ~3 lb ai/A (~0.6x the maximum seasonal rate). Applications were made at 7-day retreatment intervals; mature lettuce was harvested 21 days after final application. The registrant noted that the box containing control plants was placed close to the treatment box containing carbonyl-treated plants to monitor the uptake of radiolabeled volatile components released by treated plants. Adequate information pertaining to plant growth, maintenance, weather and screenhouse conditions, test material and application, and harvest was provided.

Mature lettuce was harvested by hand by cutting plants 1-2 inches above the soil. Whole lettuce plants were bagged and transferred to freezers. One head of lettuce from each treatment was rinsed with deionized water immediately after harvest. Samples of the rinsates were reserved for further analysis, and the rinsed lettuce heads were bagged and placed in a freezer. All samples were shipped on dry ice to the analytical laboratory, PTRL West, Inc. (Richmond, CA), on the day of harvest, and were stored frozen (<0 C) at PTRL West until processing.

Total radioactive residues (TRR)

At PTRL West lettuce samples were homogenized with dry ice and stored at ~-17 C prior to analysis. Triplicate aliquots of homogenized plant tissues were combusted and radioassayed by liquid scintillation counting (LSC). The TRRs were 3.135 ppm for S-methyl-treated plants, 1.689 ppm for carbonyl-treated plants, and 0.028 ppm for control plants. The LODs were 0.0044 ppm for S-methyl-treated plants and 0.0042 ppm for carbonyl-treated plants. The registrant stated that the low levels of radioactivity detected in control lettuce could be attributed to incorporation of $^{14}CO_2$ as a byproduct of metabolism from the carbonyl-treated lettuce plants. No further analysis was conducted on control samples.

Only 1-7 of the available radioactivity was detected in water from rinsed lettuce. No further investigations were conducted on the rinsates or on rinsed lettuce.

Extraction and hydrolysis of residues

Homogenized lettuce samples were subjected to extraction and hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Extracts were concentrated as necessary by rotary evaporation or by evaporation under a stream of nitrogen. The general extraction procedures are summarized below.

Samples of whole lettuce from each treatment were extracted three times with ACN:water (1:1, v:v), then twice each with ACN:0.1 N HCl (1:1, v:v) and ACN:0.1 N NaOH. After each extraction, the mixtures were centrifuged, and the resulting like extracts were combined. Residues were refluxed for 2.5 hours with 6 N HCl then vacuum filtered.

To determine the uptake of radioactivity into natural plant constituents, subsamples of lettuce from both treatments were extracted four times with pH 7 potassium phosphate buffer and centrifuged, and the resulting nonextractable residues were subjected to the following sequential hydrolysis steps: starch digestion with α-amylase (0.05 M potassium phosphate buffer, pH 7, 30 C, overnight); protein digestion with pronase E (0.05 M Tris buffer, pH 7.2, 25 C, 20 hours); pectin digestion via mild acid hydrolysis (0.05 M sodium acetate:0.05 M EDTA (1:1, v:v), pH 4.5, room temperature for 2 days, then 70 C for 3 hours); lignin digestion with glacial acetic acid and sodium chlorite (deionized water, 70 C, 4 hours); hemicellulose digestion with 24 aqueous potassium hydroxide (25 C, 24 hours) followed by 6 N acetic acid (25 C, 1 hour); and cellulose digestion with 72 sulfuric acid (25 C, 4 hours) followed by 24 potassium hydroxide (pH 6.5-7.5, until precipitated potassium sulfate dissolved).

The distribution of ^{14}C-activity in the extracts and hydrolysates of lettuce matrices is presented in Tables 11 and 12.

Characterization/identification of residues

Extracts and hydrolysates were analyzed by TLC and HPLC. Metabolites were identified by cochromatography with the following reference standards: acephate, methamidophos, SMPT, OMPT, DMPT, SMPAA, and OMAPAA. TLC identification of acephate was confirmed by GC/MS, TLC identification of methamidophos was confirmed by HPLC, and TLC identification of SMPT and OMAPAA was confirmed by ^{31}P-NMR following separate extraction and purification procedures.

The majority of TLC analyses were conducted on precoated silica gel (F254) plates. TLC analyses were conducted as described previously for the bean metabolism study. Additionally, for analysis of amino acids associated with some tissue degradation extracts, plates were developed with n-butanol:glacial acetic acid:water (4:1:1, v:v:v) incorporating 0.5 ninhydrin.

Reverse-phase HPLC analysis was conducted using a Supelco C-18 column and a gradient mobile phase of deionized water and ACN. Nonlabeled standards were detected by UV (215 nm), and radioactivity was quantitated by fraction collection.

The results of the ACN:water and potassium phosphate buffer extractions were comparable in terms of the amount of radioactivity released and the composition of the extractable residues as determined by TLC and HPLC. Although TLC analysis of the potassium phosphate buffer solutions of lettuce identified acephate and related metabolites, similar analyses of the remaining hydrolysates were not successful. HPLC analysis of the various hydrolysates isolated very polar and less polar products.

The registrant conducted the following additional analyses to further characterize the polar residues in the ACN:water extracts: (i) extraction and re-chromatography via TLC of isolated radiolabelled zones from the initial TLC analyses; (ii) C-18 SPE; (iii) SAX SPE followed by TLC; (iv) strong cation exchange SPE followed by TLC; (v) derivatization of polar metabolites, including Unknown P1; and (vi) oxidation using magnesium monoperoxyphthalate. These procedures essentially confirmed the findings of the initial TLC and HPLC analyses, and characterized Unknown P1 as a neutral component which does not appear to contain an oxidizable or labile [e.g., CH_3-S-P or CH_3-S-C(O)] S-methyl moiety. The registrant concluded that Unknown P1 is probably a natural product.

The distribution and characterization/identification of ^{14}C-activity in the extracts of lettuce are presented in Tables 11 and 12. A summary of the characterized and identified ^{14}C-residues is presented in Tables 13 and 14.

Table 11. Distribution and characterization/identification of radioactive residues in lettuce treated with [S-methyl-^{14}C]acephate at ~3 lb ai/A (~0.6x the maximum seasonal rate).

Fraction	% TRR	ppm	Characterization/Identification [a]
First subsample (TRR = 3.135 ppm)			
ACN:water	74.1	2.323	TLC analysis resolved: Acephate 39.4% TRR 1.234 ppm Methamidophos 8.5% TRR 0.265 ppm SMPT 8.4% TRR 0.262 ppm Unknown P1 10.1% TRR 0.318 ppm
ACN:0.1 N HCl	1.4	0.044	TLC analysis resolved: Acephate 0.2% TRR 0.007 ppm Methamidophos 0.1% TRR 0.002 ppm Unknown P1 0.9% TRR 0.027 ppm
ACN:0.1 N NaOH	5.8	0.182	TLC analysis resolved: Acephate 0.2% TRR 0.007 ppm Methamidophos 0.1% TRR 0.004 ppm Unknown P1 3.9% TRR 0.121 ppm
6 N HCl	7.5	0.235	TLC analysis resolved: Unknown P1 3.1% TRR 0.098 ppm
Nonextractable	5.7	0.179	N/A.
Second subsample (TRR = 3.135 ppm)			
Potassium phosphate buffer	89.4	2.802	Amylase hydrolysate 2.6% TRR 0.082 ppm E-Pronase hydrolysate 3.6% TRR 0.113 ppm Pectin hydrolysate1.1% TRR 0.034 ppm Lignin hydrolysate 0.8% TRR 0.025 ppm Hemicellulose hydrolysate 2.0% TRR 0.063 ppm Cellulose hydrolysate 0.4% TRR 0.013 ppm
Nonextractable	2.3	0.072	N/A.

[a] TLC identification of acephate was confirmed by GC/MS, TLC identification of methamidophos was confirmed by HPLC, and TLC identification of SMPT was confirmed by ^{31}P-NMR

Table 12. Distribution and characterization/identification radioactive residues in lettuce treated with [carbonyl-^{14}C]acephate at ~3 lb ai/A (~0.6x the maximum seasonal rate).

Fraction	% TRR	ppm	Characterization/Identification [a]
First subsample (TRR = 1.689 ppm)			
ACN:water	79.0	1.334	TLC analysis resolved: Acephate 35.2% TRR 0.595 ppm SMPT 11.5% TRR 0.195 ppm OMAPAA 23.2% TRR 0.392 ppm
ACN:0.1 N HCl	1.2	0.020	TLC analysis resolved: Acephate 0.2% TRR 0.003 ppm OMAPAA 0.6% TRR 0.010 ppm
ACN:0.1 N NaOH	2.2	0.037	TLC analysis resolved: Acephate 0.2% TRR 0.003 ppm OMAPAA 1.2% TRR 0.021 ppm
6 N HCl	6.5	0.110	TLC analysis resolved: OMAPAA 1.4% TRR 0.023 ppm
Nonextractable	5.0	0.084	N/A.
Second subsample (TRR = 1.689 ppm)			
Potassium phosphate buffer	78.7	1.330	Amylase hydrolysate 1.2% TRR 0.020 ppm E-Pronase hydrolysate 3.9% TRR 0.066 ppm Pectin hydrolysate1.1% TRR 0.019 ppm Lignin hydrolysate 0.8% TRR 0.014 ppm Hemicellulose hydrolysate 1.6% TRR 0.027 ppm Cellulose hydrolysate 0.6% TRR 0.010 ppm
Nonextractable	10.0	0.169	N/A.

[a] TLC identification of acephate was confirmed by GC/MS, and TLC identification of OMAPAA was confirmed by ^{31}P-NMR.

Table 13. Summary of characterized/identified ^{14}C-residues in lettuce treated with [S-methyl-^{14}C]acephate at ~3 lb ai/A (~0.6x the maximum seasonal rate).

Fraction	TRR = 3.135 ppm	
	% TRR	ppm
Identified [a]		
Acephate	39.8	1.248
Methamidophos	8.7	0.271
SMPT	8.4	0.262
Total identified	56.9	1.781
Characterized		
Unknown P1	18.0	0.564
Amylase hydrolysate	2.6	0.082
E-Pronase hydrolysate	3.6	0.113
Pectin hydrolysate	1.1	0.034
Lignin hydrolysate	0.8	0.025
Hemicellulose hydrolysate	2.0	0.063
Cellulose hydrolysate	0.4	0.013
Total identified/characterized	85.4	2.675
Nonextractable	2.3	0.072

[a] See Figure 1 for the chemical structures of identified metabolites.

Table 14. Summary of characterized/identified ^{14}C-residues in lettuce treated with [carbonyl-^{14}C]acephate at ~3 lb ai/A (~0.6x the maximum seasonal rate).

Fraction	TRR = 1.689 ppm	
	% TRR	ppm
Identified [a]		
Acephate	35.6	0.601
SMPT	11.5	0.195
OMAPAA	26.4	0.446
Total identified	73.5	1.242
Characterized		
Amylase hydrolysate	1.2	0.020
E-Pronase hydrolysate	3.9	0.066
Pectin hydrolysate	1.1	0.019
Lignin hydrolysate	0.8	0.014
Hemicellulose hydrolysate	1.6	0.027
Cellulose hydrolysate	0.6	0.010
Total identified/characterized	82.7	1.398
Nonextractable	10.0	0.169

[a] See Figure 1 for the chemical structures of identified metabolites.

Storage stability

All RAC samples, homogenates, and fractions were stored frozen (<0 C) prior to analysis. Analysis of lettuce extracts was initiated within 33 days of harvest. Dates of final sample analyses were not provided. The registrant submitted storage stability data demonstrating that the metabolite profile of lettuce extracted 19 days after harvest and analyzed 78 days after harvest was similar to that of lettuce extracted and analyzed ~15 months after harvest. The registrant must supply the dates of sample analyses to confirm the adequacy of the storage stability data.

Study summary

The lettuce metabolism study is acceptable pending submission of the outstanding storage stability data. Total radioactive residues were 3.135 ppm or 1.689 ppm in lettuce collected 21 days following three applications of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate, respectively, at 1 lb ai/A/application for a total application rate of 3 lb ai/A (~0.6x the maximum seasonal rate).

In lettuce from the S-methyl treatment, ~85 of TRR was characterized/identified. Acephate was identified at 39.8 of TRR (1.248 ppm), the metabolites methamidophos and SMPT were identified at 8.7 of TRR (0.271 ppm) and 8.4 of TRR (0.262 ppm), respectively. An unknown characterized as a neutral natural product comprised 18 of TRR (0.564 ppm).

In lettuce from the carbonyl treatment, ~83 of TRR was characterized/identified. Acephate was identified at 35.6 of TRR (0.601 ppm), the metabolites OMAPAA and SMPT were identified at 26.4 of TRR (0.446 ppm) and 11.5 of TRR (0.195 ppm), respectively.

Proposed metabolic pathway in plants

Based on the findings of the metabolism studies, the registrant has proposed that in plants acephate is enzyme-hydrolyzed to methamidophos, SMPT, DMPT, and OMAPAA, and that hydrolysis products, including methyl mercaptan and acetate, then enter the plant carbon pool. DMPT may also be formed via deaminolysis of methamidophos.

HED has no concerns about the metabolites, SMPAA, SMPT, DMPT, and OMAPAA. These metabolites are not cholinesterase inhibitors and it has been concluded that the effects of cholinesterase inhibition would far outweigh the toxicological significance of other effects. [Consultation with Alberto Protzel, Toxicology Branch II, 4/3/97] The residues of concern are therefore the parent, acephate and its cholinesterase inhibiting metabolite, methamidiphos.

Radiovalidation of the enforcement method

Valent has submitted radiovalidation data (1996; MRIDs 43971606, 43971607, and 44037802) for a GC/flame photometric detection (FPD) method, Valent method RM-12A-9. Method RM-12A-9 is a modification of Method I in PAM Vol. II (method RM-12A); modifications pertain to the use of wide bore capillary columns instead of packed columns for GC analysis, and a description of the use of an autosampler for analysis. Radiovalidation was conducted by Valent using radiolabeled samples from the bean, cotton, and lettuce metabolism studies.

Samples of homogenized beans, cottonseed meal, and lettuce from the [S-methyl-^{14}C]acephate treatment were combined with ethyl acetate and sodium sulfate, and filtered. The ethyl acetate extraction and filtration were repeated twice more. The resulting filtrates were combined and evaporated to dryness using a rotary evaporator. For cottonseed meal, the dried residue was partitioned with ACN:hexane (50:100, v:v), and the ACN layer was removed and washed with two aliquots of hexane, then evaporated to dryness using a rotary evaporator. The dried residue (beans, cotton, and lettuce) was then redissolved in ether, transferred to a silica gel column, and eluted with ether followed by 5 methanol in ether; the resulting eluate was evaporated to dryness, dissolved in acetone, and applied to a GC equipped with a 50 phenyl-methyl silicone column or a dimethylpolysiloxane column and an FPD. The registrant reported LODs of 0.01 ppm for acephate and 0.005 ppm for methamidophos, and limits of quantitation (LOQs) of 0.025 ppm for acephate and 0.01 ppm for methamidophos.

Sample calculations, supporting data, and representative chromatograms depicting GC analysis of acephate and methamidophos in S-methyl-treated beans, cottonseed meal, and lettuce were provided. The radiovalidation data comparing the results of the GC/FPD analysis to data obtained from the bean, cotton, and lettuce metabolism studies are presented in Table 15. Concurrent method recoveries from samples of untreated commodities fortified with acephate and methamidophos are also presented in Table 15. The registrant reported that the analysis was repeated for lettuce because the method recovery for methamidophos in the first analysis exceeded 120 , untreated lettuce from the metabolism study was not used for the data set, and there was a possibility of non-homogeneous sampling from aliquotting the lettuce sample.

Table 15. Determination of acephate and methamidophos in samples of S-methyl-treated beans, cottonseed meal, and lettuce from the metabolism studies using TLC radiochemical analysis and the GC/FPD enforcement method.

Commodity	Method	Acephate, ppm	Methamidophos, ppm
Beans	TLC Radiochemical Analysis	2.236	0.915 [a]
	Method RM-12A-9	2.57	0.879
Cottonseed meal	TLC Radiochemical Analysis	0.025	0.011 [b]

	Method RM-12A-9	**0.013**	**<0.005**
Lettuce	**TLC Radiochemical Analysis**	**1.248**	**0.209** [c]
	Method RM-12A-9	**0.888, 0.949**	**0.161, 0.170**
Concurrent Method Recovery [d]			
Beans	**Method RM-12A-9**	**118**	**107**
Cottonseed meal	**Method RM-12A-9**	**103**	**113**
Lettuce	**Method RM-12A-9**	**118, 120**	**114, 123**

[a] **Equivalent to 1.187 ppm acephate equivalents.**
[b] **Equivalent to 0.014 ppm acephate equivalents.**
[c] **Equivalent to 0.271 ppm acephate equivalents.**
[d] **Samples of beans were fortified with acephate and methamidophos at 2.5 and 1.0 ppm, respectively, samples of cottonseed meal were fortified at 0.025 and 0.010 ppm, respectively, and samples of lettuce were fortified at 1.0 and 0.40 ppm, respectively.**

The submitted radiovalidation data for enforcement method RM-12A-9 are adequate. The method adequately recovered residues of acephate and methamidophos from samples of beans and lettuce treated with [S-methyl-^{14}C]acephate (from the bean and lettuce metabolism studies). In addition, the method adequately recovered residues of acephate at levels near the LOQ from samples of cottonseed meal treated with [S-methyl-^{14}C]acephate (from the cotton metabolism study); residues of methamidophos were below the LOQ in the sample, and were not detected.

Qualitative Nature of the Residue in Animals

Ruminants

In-life phase

Valent submitted data (1996; MRID 43971604) depicting the metabolism of [S-methyl-^{14}C]acephate and [carbonyl-^{14}C]acephate in lactating goats. The in-life and analytical phases of the study were conducted by Ricerca, Inc. (Painesville, OH). Gelatin dose capsules were prepared from [S-methyl-^{14}C]acephate (specific activity 51 mCi/mmol, radiochemical purity 99.4) and [carbonyl-^{14}C]acephate (specific activity 52.1 mCi/mmol, radiochemical purity 98.7). Two lactating goats were dosed orally with [^{14}C]acephate (one with the S-methyl-labeled test substance and one with the carbonyl-labeled test substance) using a balling gun twice daily for 3 consecutive days at 15 ppm. The dosing level of 15 ppm is equivalent to ~0.4x the maximum theoretical dietary burden; see Table 16 for calculation of dietary burden. The registrant based the dosing level on the highest established acephate tolerance. We note that the dietary burden calculation is approximate because magnitude of the residue data remain outstanding for the listed feed items. A third goat was dosed with a placebo to serve as a control. The goats were housed in stanchions equipped with Plexiglass hoods from which air was drawn through potassium hydroxide solutions to trap expired air; the registrant stated that expired air was trapped as a precaution to prevent exposure of study personnel to volatile compounds containing ^{14}C.

Table 16. Calculation of maximum theoretical dietary burden of acephate to beef and dairy cattle.

Feed item	% Dry matter	Tolerance	% in Diet	Dietary burden, ppm
Cottonseed	**88**	**2**	**25**	**0.57**
Cottonseed, meal	**89**	**8**	**15**	**1.348**
Grass, forage	**25**	**15**	**60**	**36**
Total			**100**	**37.92**

During the testing period, the goats were fed Rumilab Chow® and a milking ration; the goats were allowed water *ad libitum*. The registrant provided sufficient descriptions of preparation of dose solutions and capsules and animal husbandry practices, and data concerning daily feed intake, body weights, and milk production. Milk was collected twice daily prior to dosing; milk samples were stored frozen following collection. Goats were sacrificed 20 hours after the last dose, and the following samples were collected: blood, kidneys, heart, liver, muscle (composite of loin, rear leg, and foreleg), fat (composite of omental and perirenal), and gastrointestinal tract and contents. Samples were stored at <-15 C until analysis.

Total radioactive residues (TRR)

Triplicate aliquots of homogenized tissues were analyzed by LSC following combustion; milk samples were radioassayed directly by LSC. The TRR found in tissues and milk of the test goats are presented in Table 17. The LOQs were 0.017 and 0.016 ppm, respectively, for S-methyl- and carbonyl-labeled [^{14}C]acephate in fat; 0.0083 and 0.0078 ppm, respectively, in other tissues; and 0.0013 and 0.0012 ppm, respectively, in milk.

Table 17. Total radioactive residues found in milk and tissues from lactating goats following administration of S-methyl- or carbonyl-labeled [^{14}C]acephate for 3 days.

	TRR, ppm [^{14}C]acephate equivalents	
Matrix	**S-Methyl-label**	**Carbonyl-label**
Milk: Day 1 pm	**0.2349**	**0.2074**
Day 2 am	**0.5210**	**0.6077**
Combined	**0.4404**	**0.4973**
Day 2 pm	**0.5769**	**0.7138**
Day 3 am	**0.5085**	**0.6650**
Combined	**0.5290**	**0.6756**
Day 3 pm	**0.5503**	**0.7362**
Day 4 am	**0.4982**	**0.6571**
Combined	**0.5128**	**0.6787**
Fat	**0.018**	**0.103**
Muscle	**0.168**	**0.200**
Kidney	**0.502**	**0.358**
Liver	**1.085**	**1.042**

Samples of urine, feces, and cage washes were also collected and analyzed for TRR; the registrants provided additional data which indicated that 46.85 , 5.23 , and 1.08 of the administered [S-methyl-^{14}C]acephate dose was eliminated in the urine, feces, and cage washes, respectively, and 63.48 , 7.90 , and 1.44 of the administered [carbonyl-^{14}C]acephate dose was eliminated in the urine, feces, and cage washes, respectively.

Extraction and hydrolysis of residues

Milk and tissue samples were subjected to extraction and/or hydrolysis procedures for residue characterization and identification. The registrants provided adequate descriptions of the fractionation schemes used for the analysis of acephate residues in goat milk and tissues. A flow chart of the extraction scheme was provided for each matrix. The general extraction and fractionation procedures for goat milk and tissues are summarized below.

Subsamples of milk (Day 2 combined) were mixed with ACN and centrifuged. The supernatant was concentrated by rotary evaporation, diluted with deionized water, and further concentrated. The concentrated extract was centrifuged, filtered, and reserved for HPLC analysis.

A second set of milk subsamples were separated into cream and skim milk by centrifugation of cooled milk (4 C). Casein was precipitated from skim milk by acidification to pH 4.6 (1 M HCl) followed by centrifugation; the remaining fraction was whey. Both casein and whey were freeze dried prior to any further analysis. Casein was mixed with water and centrifuged. The aqueous supernatant was filtered and reserved for HPLC analysis. Freeze-dried whey was dissolved in water and analyzed by HPLC and TLC. The whey was then hydrolyzed with 1 M HCl (80 C for 3 hours) and analyzed by HPLC.

Cream samples were extracted with dichloromethane (DCM) and water. Any solids which formed were removed and the DCM and water were allowed to separate. The aqueous portion was extracted with DCM, which was combined with the first DCM extract.

A subsample of fat was sequentially extracted with hexane, DCM, and ethyl acetate. The combined organic extracts were partitioned with deionized water, and the aqueous and organic phases were separately analyzed.

Tissue samples (liver, kidney, and muscle) were extracted twice with a buffer solution prepared by mixing 0.1 M potassium dihydrogen phosphate, 0.1 M sodium hydroxide, and deionized water (50:29:21, v:v:v). The extracts were centrifuged and the aqueous supernatants were diluted with ACN to precipitate proteins, which were isolated by centrifugation (protein fraction). The remaining solids after buffer extraction were extracted twice with ACN:water (1:1, v:v) and centrifuged; the extracts were combined with the aqueous extracts remaining after protein precipitation. The remaining solids were then extracted with ethyl acetate. The combined aqueous extracts were extracted twice with hexane; the hexane extracts were combined with the ethyl acetate extract and the aqueous extracts were extracted twice with ethyl ether. The ether extracts were combined with the previous organic extracts and concentrated (nonpolar fraction). The aqueous extracts (polar fraction) were rotary evaporated to remove residual organic solvent and reserved for HPLC analysis. The registrant stated that the nonpolar fraction would consist

predominantly of ^{14}C-labeled lipids, and that the polar fraction would consist of ^{14}C-acephate and related ^{14}C-organophosphorus compounds as well as polar ^{14}C-labeled natural products.

The remaining nonextractable residues of tissues were digested with protease (0.1 M phosphate buffer, pH 7, 37 C, overnight) and centrifuged. The hydrolysate was analyzed by HPLC. The hydrolysate was also lyophilized and sequentially extracted with ethyl acetate and ethyl acetate:methanol (8:1, v:v); the remaining solids were dissolved in water.

The distribution of total radioactive residues in the extracts of [S-methyl-^{14}C]acephate- and [carbonyl-^{14}C]acephate-dosed goat matrices are presented in Tables 18 and 19, respectively.

Characterization and identification of ^{14}C-residues

Extracts and hydrolysates were analyzed by HPLC and TLC. The following HPLC methods were used: Method 1, a C-18 column and a gradient mobile phase of methanol, water, ACN, and 0.5 mM heptatriethyl ammonium phosphate in 10 mM potassium dihydrogen phosphate; Method 2, a SPLC 18-DB column and a gradient mobile phase of methanol and water containing 0.05 phosphoric acid and 1.5 isopropanol; Method 3, a Nucleosil 5N$(CH_3)_2$ column and a gradient mobile phase of ACN and 0.01 M ammonium chloride (pH 7.0); and Method 4, an LC-NH_2 column and an isocratic mobile phase of ACN and water. Nonlabeled standards were detected by UV (220 or 230 nm), and radioactivity was detected using a radioisotope detector. Metabolites were identified by cochromatography or comparison of retention times with those of the following reference standards: acephate, methamidophos, acetamide, SMPT, SMPAA, OMPT, OMAPAA, DMPT, lactose, galactose, and glucose, as well as the fatty acids palmitic acid, oleic acid, pyristic acid, n-caproic acid, linoleic acid, caprylic acid, and n-capric acid. Method 1 was used for the analysis of aqueous, ACN, and polar extracts and aqueous hydrolysates of milk and tissues. Method 2 was used for the identification of fatty acids in cream and fat. Method 3 was used for the identification of OMAPAA, SMPT, and SMPAA in liver and kidney. Method 4 was used for the identification of lactose, galactose, and glucose in whey.

TLC analyses were conducted on silica gel (60A LK6F) plates using the following solvent systems: Method 1, ethanol:water (95:5, v:v); and Method 2, isopropanol:acetone:0.2 M boric acid (3:5:1, v:v:v). Nonlabeled standards were visualized by spraying with 1 ammonium molybdate and 1 stannous chloride in 10 HCl. For Method 3 only, standards were visualized by spraying with sulfuric acid and heating the plate until the sugars were charred. Radioactivity was detected and quantitated using a linear analyzer. Method 1 was used to analyze polar tissue extracts. Method 2 was used for the analysis of lactose in whey.

The identification of acephate in tissues was confirmed by TLC and GC/MS in samples of S-methyl-treated kidney and carbonyl-treated muscle, respectively.

Because the HPLC retention times of OMAPAA, DMPT, and SMPAA were such that they could not be separated from polar natural products, the polar fractions from kidney and liver were subjected to dialysis, Spectra/Por MWCO 500 amu tubing); dialyzed residues (<500 amu) were lyophilized. The dried residues were sequentially dissolved with ethanol:water (95:5, v:v) and deionized water. The ethanol:water fraction, which was found to contain the majority of the radioactivity, was analyzed by HPLC and TLC. Prior to HPLC analysis, the sample was rotary evaporated to dryness and redissolved in ACN:water (6:1, v:v). Prior to TLC analysis, the sample was cleaned on a silica gel column. Identification of the metabolites by HPLC was confirmed by TLC.

To characterize/identify the protein fraction of liver, the aqueous buffer extracts, prior to the addition of ACN, were subjected to dialysis to separate high molecular weight compounds (>12,000 to 14,000 amu); the fraction containing high molecular weight compounds was found to comprise a large portion of residues (~25 TRR). HPLC analyses of the <12,000-14,000-amu fraction were compared to HPLC analyses of a sample which had not undergone dialysis and from which proteins had been precipitated to demonstrate that low molecular weight organophosphate compounds did not coprecipitate with the proteins.

To characterize radioactivity in cream, the DCM-soluble ^{14}C-residues from the cream of the [carbonyl-^{14}C]acephate-dosed goat were saponified in ethanolic KOH (60-80 C for 3 hours). The resulting mixture was diluted with deionized water, acidified with HCl, and extracted with DCM. The DCM extract was concentrated by rotary evaporation and dissolved in methanol:water (2:1, v:v) for separation by HPLC. Regions corresponding to the retention times of capric and palmitic acid were isolated, and the p-bromophenacyl ester derivatives of the isolated fatty acids as well as of reference standards of capric acid and palmitic acid were prepared (Durst method) and analyzed by LC/MS and HPLC . The chromatographic analyses indicated that [^{14}C]capric acid and [^{14}C]palmitic acid were present in the cream sample. No quantitative data for these analyses were presented.

To characterize/identify radioactivity in fat, a second subsample of fat ([carbonyl-^{14}C]acephate-treated sample only) was extracted twice with methanol:DCM and methanol, water, and DCM were added to the combined extracts. After phase separation, the DCM phase was isolated and rotary evaporated to dryness. The dried residue was saponified with 0.5 M potassium hydroxide in ethanol:water (3:1, v:v; 80 C for 2 hours). Following acidification with 3 M HCl and dilution with deionized water, the fatty acids were partitioned into DCM. The DCM partitions were rotary evaporated to dryness and the residue redissolved in methanol and water for analysis by HPLC. HPLC analysis indicated a distribution of residues similar to that for the saponifiable

^{14}C-residues of cream, and radioactivity eluting at the retention times of capric, myristic, and palmitic acids. Because of problems with column overloading, no quantitative data were presented.

Summaries of the characterized and identified residues found in goat matrices following dosing with [S-methyl-^{14}C]acephate and [carbonyl-^{14}C]acephate are presented in Tables 20 and 21, respectively.

Table 18. Distribution of total radioactive residues in milk and tissues from a lactating goat orally dosed with [S-methyl-^{14}C]acephate at 15 ppm in the diet (~0.4x the maximum theoretical dietary burden) for 3 days.

Fraction	% TRR	ppm	Characterization/Identification
Milk (Day 2 combined; TRR = 0.529 ppm) - 1st subsample			
ACN	23.3	0.123	HPLC analysis resolved: Acephate 14.6% TRR 0.077 ppm Methamidophos 1.0% TRR 0.005 ppm SMPT 0.1% TRR <0.001 ppm Unknown (7 min.) 6.5% TRR 0.035 ppm
Nonextractable	N/R [a]	N/R	Not further analyzed (N/A).
Milk (Day 2 combined; TRR = 0.529 ppm) - 2nd subsample			
Cream	12.3	0.065	Extracted with DCM and water.
DCM	6.4	0.034	N/A.
Aqueous	3.0	0.016	N/A.
Solids	N/D [b]	N/D	This fraction was not radioassayed.
Skim milk	87.7	0.464	Adjusted to pH 4.6 to precipitate casein.
Casein	36.5	0.193	Freeze dried and then mixed with water.
Aqueous	8.5	0.045	HPLC analysis resolved: Acephate 1.5% TRR 0.008 ppm Methamidophos 0.1% TRR 0.001 ppm SMPT 0.4% TRR 0.002 ppm Plus early-eluting fractions (6.3% TRR, 0.034 ppm) the registrant attributed to soluble proteins.
Solids	N/D	N/D	This fraction was not radioassayed. The registrant calculated (by difference) that this fraction accounted for 28.0% TRR (0.148 ppm) and attributed it to denatured proteins.
Whey	51.2	0.271	HPLC analysis resolved: Lactose 32.9% TRR 0.174 ppm HPLC analysis of an acid-hydrolyzed sample indicated the presence of [^{14}C]galactose and [^{14}C]glucose, the acid hydrolysis products of lactose. TLC analyses confirmed the identity of lactose. HPLC analysis resolved: Acephate 5.2% TRR 0.028 ppm Methamidophos 0.7% TRR 0.004 ppm SMPT 6.6% TRR 0.035 ppm Plus early-eluting fractions (37.4% TRR, 0.198 ppm) the registrant attributed to natural products (lactose and soluble peptide/protein).
Fat (TRR = 0.018 ppm)			
Organic	51.4	0.009	N/A.

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification
Aqueous	18.9	0.003	N/A.
Nonextractable	32.8	0.006	N/A.
Muscle (TRR = 0.168 ppm)			
Polar fraction	61.3	0.103	HPLC analysis resolved: Acephate 26.2% TRR 0.044 ppm Methamidophos <6.0% TRR <0.01 ppm Unknown (7 min.) 16.2% TRR 0.027 ppm Unknown (9 min.) 8.2% TRR 0.014 ppm
Nonpolar fraction	3.4	0.006	N/A.
Proteins	14.2	0.024	N/A.
Nonextractable	24.1	0.040	N/A.
Kidney (TRR = 0.502 ppm)			
Polar fraction	39.8	0.200	HPLC analysis resolved: Acephate 13.9% TRR 0.070 ppm Methamidophos <2.0% TRR <0.01 ppm Unknown (7 min.) 22.6% TRR 0.113 ppm In addition, a subsample was subjected to dialysis, and the dialyzed residues were extracted (as described in the text). TLC analysis resolved: SMPAA5.6% TRR 0.028 ppm
Nonpolar fraction	6.6	0.033	N/A.
Proteins	28.3	0.142	N/A.
Nonextractable	20.3	0.102	Subjected to protease digestion.
Protease hydrolysate	17.7	0.089	N/A.
Nonextractable	5.6	0.028	N/A.
Liver (TRR = 1.085 ppm)			
Polar fraction	23.0	0.250	HPLC analysis resolved: Acephate 4.2% TRR 0.046 ppm Methamidophos <0.9% TRR <0.01 ppm Unknown (7 min.) 16.0% TRR 0.174 ppm In addition, a subsample was subjected to dialysis, and the dialyzed residues were extracted (as described in the text). TLC analysis resolved: SMPT0.6% TRR 0.006 ppm SMPAA1.3% TRR 0.014 ppm
Nonpolar fraction	4.2	0.046	N/A.
Proteins	45.4	0.493	This fraction was not further analyzed. Dialysis of a subsample of buffer extracts indicated that 25.1% TRR (0.272 ppm) consisted of high molecular weight compounds (>12,000-14,000 amu).
Nonextractable	15.4	0.167	Subjected to protease digestion.
Protease hydrolysate	11.2	0.122	N/A.

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification
Nonextractable	4.4	0.048	N/A.

[a] N/R = Not reported. The registrant indicated that this fraction was radioassayed (or combusted and radioassayed); however no LSS results were reported.
[b] N/D = Not determined.

(continued; footnotes follow)

Table 19. Distribution of total radioactive residues in milk and tissues from a lactating goat orally dosed with [carbonyl-^{14}C]acephate at 15 ppm in the diet (~0.4x the maximum theoretical dietary burden) for 3 days.

Fraction	% TRR	ppm	Characterization/Identification
Milk (Day 2 combined; TRR = 0.676 ppm) - 1st subsample			
ACN	16.7	0.113	HPLC analysis resolved: Acephate 11.6% TRR 0.078 ppm Acetamide 3.3% TRR 0.022 ppm SMPT and/or 0.05% TRR <0.001 ppm OMPT 0.02% TRR <0.001 ppm Unknown (7 min.) 1.2% TRR 0.008 ppm
Nonextractable	N/R [a]	N/R	Not further analyzed (N/A).
Milk (Day 2 combined; TRR = 0.676 ppm) - 2nd subsample			
Cream	69.4	0.469	Extracted with DCM and water.
DCM	55.6	0.376	Saponified and extracted with DCM; preparative HPLC isolated regions corresponding to the retention times of capric and palmitic acids (fatty acids). HPLC and LC/MS analysis of the p-bromophenacyl derivatives of the isolated regions confirmed the incorporation of radioactivity into capric and palmitic acid. No quantitative data were presented.
Aqueous	0.7	0.005	N/A.
Solids	N/D	N/D	This fraction was not radioassayed.
Skim milk	30.6	0.207	Adjusted to pH 4.6 to precipitate casein.
Casein	10.9	0.074	Freeze dried and then mixed with water.
Aqueous	2.7	0.018	HPLC resolved: Acephate 0.9% TRR 0.006 ppm SMPT and/or 0.2% TRR 0.002 ppm OMPT 0.08% TRR 0.001 ppm Plus early-eluting fractions (1.2% TRR, 0.008 ppm) the registrant attributed to soluble proteins.
Solids	N/D [b]	N/D	This fraction was not radioassayed. The registrant calculated (by difference) that this fraction accounted for 8.2% TRR (0.056 ppm) and attributed it to denatured proteins.
Whey	19.6	0.132	HPLC analysis resolved: Lactose 4.0% TRR 0.027 ppm HPLC analysis of an acid-hydrolyzed sample indicated the presence of [^{14}C]galactose and [^{14}C]glucose, the acid hydrolysis products of lactose. TLC analyses confirmed the identity of lactose. HPLC analysis resolved: Acephate 3.1% TRR 0.021 ppm SMPT and/or 1.0% TRR 0.007 ppm OMPT 4.6% TRR 0.031 ppm Plus early-eluting fractions (10.3% TRR, 0.070 ppm) the registrant attributed to natural products (lactose and

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification
			soluble peptide/protein).
Fat (TRR = 0.103 ppm)			
Organic	85.3	0.088	Not further analyzed. A second subsample of fat was extracted (as described in the text) and the organosoluble residues were saponified and extracted into DCM. HPLC analysis of the DCM extract indicated radioactivity eluting at the retention times of capric, myristic, and palmitic acids. Due to problems with column overloading, useful quantitative data were not available.
Aqueous	4.5	0.005	N/A.
Nonextractable	3.0	0.003	N/A.
Muscle (TRR = 0.200 ppm)			
Polar fraction	70.7	0.141	HPLC analysis resolved: Acephate 21.5% TRR 0.043 ppm Unknown (7 min.) 37.3% TRR 0.074 ppm
Nonpolar fraction	5.5	0.011	N/A.
Proteins	5.9	0.012	N/A.
Nonextractable	10.0	0.020	N/A.
Kidney (TRR = 0.358 ppm)			
Polar fraction	73.0	0.261	HPLC analysis resolved: Acephate 25.7% TRR 0.092 ppm Unknown (7 min.) 42.9% TRR 0.153 ppm In addition, a subsample was subjected to dialysis, and the dialyzed residues were extracted (as described in the text). TLC analysis resolved: OMAPAA 10.3% TRR 0.037 ppm SMPT 1.7% TRR 0.006 ppm
Nonpolar fraction	13.5	0.048	N/A.
Proteins	9.7	0.035	N/A.
Nonextractable	5.8	0.021	Subjected to protease digestion.
Protease hydrolysate	4.7	0.017	N/A.
Nonextractable	0.8	0.003	N/A.
Liver (TRR = 1.042 ppm)			
Polar fraction	34.6	0.361	HPLC analysis resolved: Acephate 4.8% TRR 0.050 ppm Acetamide 4.5% TRR 0.047 ppm Unknown (7 min.) 27.5% TRR 0.286 ppm In addition, a subsample was subjected to dialysis, and the dialyzed residues were extracted (as described in the text). TLC analysis resolved: SMPT 2.5% TRR 0.026 ppm OMAPAA 6.9% TRR 0.072 ppm
Nonpolar fraction	2.2	0.023	N/A.
Proteins	44.5	0.464	This fraction was not further analyzed. Dialysis of a subsample of buffer extracts indicated that 26.8% TRR

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification
			(0.279 ppm) consisted of high molecular weight compounds (>12,000-14,000 amu).
Nonextractable	10.9	0.114	Subjected to protease digestion.
Protease hydrolysate	10.6	0.110	HPLC analysis indicated that a large portion of the radioactivity eluted in the region of acephate. However, TLC analysis indicated that acephate was not present.
Nonextractable	1.0	0.010	N/A.

[a] N/R = Not reported. The registrant indicated that this fraction was radioassayed (or combusted and radioassayed); however no LSS results were reported.
[b] N/D = Not determined.

(continued; footnotes follow)

Table 20. Summary of radioactive residues characterized/identified in milk and tissues from a lactating goat orally dosed with [S-methyl-^{14}C]acephate at 15 ppm (~0.4x the maximum theoretical dietary burden) in the diet for 3 days.

	Milk (TRR = 0.529 ppm)		Fat (TRR = 0.018 ppm)		Muscle (TRR = 0.168 ppm)		Kidney (TRR = 0.502 ppm)		Liver (1.085 ppm)	
Fraction	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**
Identified [a]										
Acephate	14.6	0.077	--	--	26.2	0.044	13.9	0.070	4.2	0.046
Methamidophos	1.0	0.005	--	--	<6.0	<0.01	<2.0	<0.01	<0.9	<0.01
SMPT	7.0	0.037	--	--	--	--	--	--	0.6	0.006
Lactose	32.9	0.174	--	--	--	--	--	--	--	--
SMPAA	--	--	--	--	--	--	5.6	0.028	1.3	0.014
Total identified	55.5	0.294	--	--	<32.2	<0.054	<21.5	<0.108	<7.0	<0.076
Characterized										
Early-eluting fractions [b]	10.8	0.057	--	--	24.4	0.041	17.0	0.085	14.7	0.160
Organic	6.4	0.034	51.4	0.009	--	--	--	--	--	--
Aqueous	3.0	0.016	18.9	0.003	--	--	--	--	--	--
Nonpolar fraction	--	--	--	--	3.4	0.006	6.6	0.033	4.2	0.046
Protein fraction [c]	--	--	--	--	14.2	0.024	28.3	0.142	45.4	0.493
Protease hydrolysate	--	--	--	--	--	--	17.7	0.089	11.2	0.122
Total identified/ characterized	75.7	0.401	70.3	0.012	<74.2	<0.125	<91.1	<0.457	<82.5	<0.897
Nonextractable	N/R [d]	N/R	32.8	0.006	24.1	0.040	5.6	0.028	4.4	0.048

[a] **See Figure 1 for chemical structures of identified metabolites.**

[b] **Radioactivity in polar/aqueous fractions eluting from HPLC within retention times of 6-9 minutes; this fraction may have been adjusted by the study reviewer for radioactivity accounted for by lactose and SMPAA (which eluted within that retention time window).**

[c] **The registrant termed this fraction, which was precipitate formed after addition of ACN to aqueous extracts, the protein fraction. For liver, dialysis indicated that 25.1% TRR (0.272 ppm) consisted of high molecular weight compounds (>12,000-14,000 amu).**

[d] **N/R = not reported.**

Table 21. Summary of radioactive residues characterized/identified in milk and tissues from a lactating goat orally dosed with [carbonyl-^{14}C]acephate at 15 ppm (~0.4x the maximum theoretical dietary burden) in the diet for 3 days.

Fraction	Milk (TRR = 0.676 ppm)		Fat (TRR = 0.103 ppm)		Muscle (TRR = 0.200 ppm)		Kidney (TRR = 0.358 ppm)		Liver (TRR = 1.042 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]										
Acephate	11.6	0.078	--	--	21.5	0.043	25.7	0.092	4.8	0.050
Acetamide	3.3	0.022	--	--	--	--	--	--	4.5	0.047
SMPT and/or	1.2	0.009	--	--	--	--	1.7	0.006	2.5	0.026
OMPT	4.7	0.032	--	--	--	--	--	--	--	--
Lactose	4.0	0.027	--	--	--	--	--	--	--	--
OMAPAA	--	--	--	--	--	--	10.3	0.037	6.9	0.072
Total identified	24.8	0.168	--	--	21.5	0.043	37.7	0.135	18.7	0.195
Characterized										
Early-eluting fractions [b]	7.5	0.051	--	--	37.3	0.074	32.6	0.116	20.6	0.214
Organic	55.6 [c]	0.376	85.3 [d]	0.088	--	--	--	--	--	--
Aqueous	0.7	0.005	4.5	0.005	--	--	--	--	--	--
Nonpolar fraction	--	--	--	--	5.5	0.011	13.5	0.048	2.2	0.023
Protein fraction [e]	--	--	--	--	5.9	0.012	9.7	0.035	44.5	0.464
Protease hydrolysate	--	--	--	--	--	--	4.7	0.017	10.6	0.110
Total identified/ characterized	88.6	0.599	89.8	0.093	70.2	0.140	98.2	0.351	96.6	1.006
Nonextractable	N/R [f]	N/R	3.0	0.003	10.0	0.020	0.8	0.003	1.0	0.010

[a] See Figure 1 for chemical structures of identified metabolites.

[b] Radioactivity in polar/aqueous fractions eluting from HPLC within retention times of 6-9 minutes; this fraction may have been adjusted by the study reviewer for radioactivity accounted for by lactose and OMAPAA (which eluted within that retention time window).

[c] This extract was saponified, extracted, derivatized, and analyzed by HPLC and LC/MS which confirmed the incorporation of radioactivity into capric and palmitic acids; no quantitative data were presented.

[d] A second subsample was saponified, extracted, and analyzed by HPLC which indicated radioactivity eluting at the retention times of capric, myristic, and palmitic acid; no quantitative data were presented..

[e] **The registrant termed this fraction, which was precipitate formed after addition of ACN to aqueous extracts, the protein fraction. For liver, dialysis indicated that 26.8% TRR (0.279 ppm) consisted of high molecular weight compounds (>12,000-14,000 amu).**

[f] **N/R = not reported.**

Storage stability

All samples were stored frozen (<-15 C) until extraction and analysis. Samples were stored for up to 209 days (~7 months) between collection and final analysis; all analyses except those for acetamide were conducted within 5 months of sample collection. The registrant stated that because ^{14}C-acephate was incorporated into natural products, [^{14}C]acephate was the only residue for which quantitative storage stability data could be generated. To evaluate storage stability, the registrant analyzed a sample of milk (S-methyl label, day 2) early in the study and reanalyzed the sample approximately 4 months later; the reanalysis indicated that neither acephate nor methamidophos degraded during storage. In addition, a sample of liver (carbonyl label) which had initially been analyzed approximately 4 months after collection was reanalyzed approximately 3 months later. The reanalysis indicated that residues of acephate did not degrade over 3 months of storage.

Because the majority of analyses were conducted within 5 months of sample collection and because the registrant provided information demonstrating that residues of acephate are stable for approximately 4 months in milk and liver, CBRS concludes that no additional storage stability data are required to support this study.

Study summary

The qualitative nature of the residue in ruminants is adequately understood. Following oral administration of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate to lactating goats for 3 days at 15 ppm (~0.4x the maximum dietary burden), the TRR, respectively, were 0.2349-0.5769 ppm and 0.2074-0.7362 ppm in milk, 0.018 ppm and 0.103 ppm in fat, 0.168 ppm and 0.200 ppm in muscle, 0.502 ppm and 0.358 ppm in kidney, and 1.085 ppm and 1.042 ppm in liver.

The majority of ^{14}C-residues, ~70-98 , were characterized/identified in milk, fat, muscle, kidney, and liver. Following dosing with the S-methyl-labeled test substance, acephate was identified in milk (14.6 TRR, 0.077 ppm), muscle (26.2 TRR, 0.044 ppm), kidney (13.9 TRR, 0.070 ppm), and liver (4.2 TRR, 0.046 ppm). Methamidophos was identified in milk (1.0 TRR, 0.005 ppm) and was detected in muscle, kidney, and liver at levels <0.01 ppm. Other identified metabolites included SMPT (7.0 TRR, 0.037 ppm in milk; and 0.6 TRR, 0.006 ppm in liver), and SMPAA (5.6 TRR, 0.028 ppm in kidney; and 1.3 TRR, 0.014 ppm in liver). The registrant demonstrated the incorporation of radioactivity into natural products by identifying [^{14}C]lactose (32.9 TRR, 0.174 ppm) in milk and by showing that 25.1 of liver TRR (0.272 ppm) consisted of high molecular weight compounds (>12,000 amu). No metabolites were identified in fat; however, TRR in fat were low (0.018 ppm).

Following dosing with the carbonyl-labeled test substance, acephate was identified in milk (11.6 TRR, 0.078 ppm), muscle (21.5 TRR, 0.043 ppm), kidney (25.7 TRR, 0.092 ppm), and liver (4.8 TRR, 0.050 ppm). Acetamide was identified in milk (3.3 TRR, 0.022 ppm) and liver (4.5 TRR, 0.047 ppm). Other identified metabolites included SMPT (1.2 TRR, 0.009 ppm in milk plus an additional 4.7 TRR, 0.032 ppm which was either SMPT or OMPT; 1.7 TRR, 0.006 ppm in kidney; and 2.5 TRR, 0.026 ppm in liver), and OMAPAA (10.3 TRR, 0.037 ppm in kidney; and 6.9 TRR, 0.072 ppm in liver). The registrant demonstrated the incorporation of radioactivity into natural products by identifying [^{14}C]lactose (4.0 TRR, 0.027 ppm) in milk, by identifying [^{14}C]capric acid and [^{14}C]palmitic acid in cream, and by showing that 26.8 of liver TRR (0.279 ppm) consisted of high molecular weight compounds (>12,000 amu). No metabolites were identified in fat; however, HPLC analyses indicated radioactivity eluting at the retention times of capric, myristic, and palmitic acids.

Poultry

In-life phase

Valent submitted data (1996; MRID 44037803) depicting the metabolism of [S-methyl-^{14}C]acephate and [carbonyl-^{14}C]acephate in laying hens. The in-life and analytical phases of the study were conducted by Ricerca, Inc. (Painesville, OH). Gelatin dose capsules were prepared from [S-methyl-^{14}C]acephate (specific activity 51 mCi/mmol, radiochemical purity 99.4) and [carbonyl-^{14}C]acephate (specific activity 52.1 mCi/mmol, radiochemical purity 98.7). Seven laying hens were dosed orally with [S-methyl-^{14}C]acephate twice daily for 3 consecutive days at a total of 10 ppm in the diet. An additional seven hens were dosed with [carbonyl-^{14}C]acephate in the same manner, and four hens were dosed with a placebo to serve as controls. The daily dose was equivalent to ~5x the maximum theoretical dietary burden; see Table 22 for calculation of dietary burden. We note that the dietary burden calculation is approximate because magnitude of the residue data remain outstanding for the listed feed items. The hens were housed in individual cages equipped with Plexiglass hoods from which air was drawn through potassium hydroxide solutions to trap expired air; the registrant stated that expired air was trapped as a precaution to prevent exposure of study personnel to volatile compounds containing ^{14}C.

Table 22. Calculation of maximum theoretical dietary burden of acephate to poultry.

Feed item	Tolerance	% in Diet	Dietary burden, ppm
Cottonseed meal	**8.0**	**20**	**1.60**
Cowpea seed	**3.0**	**10**	**0.30**
Peanut meal	**0.2**	**25**	**0.05**

Total	**1.95**

During the testing period, the hens were allowed water and a commercial feed mixture *ad libitum*. The registrant provided sufficient descriptions of preparation of dose solutions and capsules and animal husbandry practices, and data concerning daily feed intake, body weights, and egg production. Eggs were collected once daily. Hens were sacrificed ~20 hours after the last dose, and the following samples were collected: blood, liver, muscle (combination of breast and thigh), fat (abdominal and skin with fat), gastrointestinal tract and contents, ovaries and oviduct contents. Samples were stored at <-15 C until analysis.

Total radioactive residues (TRR)

Tissues were homogenized in the presence of dry ice prior to TRR analysis; eggs were separated into whites and yolks. Egg and tissue samples were analyzed in triplicate by LSC following combustion. The TRR found in eggs and tissues of test hens are presented in Table 23. The LOQs were 0.012 and 0.010 ppm, respectively, for S-methyl- and carbonyl-labeled [^{14}C]acephate in egg white; 0.027 and 0.021 ppm, respectively, in egg yolk; and 0.009 and 0.008 ppm, respectively, in other tissues. The registrant provided additional data which indicate that 72.3 and 5.90 of the applied dose was eliminated in the excreta and expired (collected in traps), respectively, for the [S-methyl-^{14}C]acephate-dosed hens, and 44.5 and 16.0 was excreted and expired, respectively, for the [carbonyl-^{14}C]acephate-dosed hens.

Table 23. Total radioactive residues found in eggs and tissues from laying hens following administration of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate for 3 days.

Matrix		TRR, ppm [^{14}C]acephate equivalents	
		S-Methyl-label	Carbonyl-label
Egg white:	**Day 1**	**0.08**	**0.02**
	Day 2	**0.19**	**0.18**
	Day 3	**0.34**	**0.31**
Egg yolk:	**Day 1**	**0.02**	**<0.021**
	Day 2	**0.05**	**0.08**
	Day 3	**0.17**	**1.52**
Whole eggs (Day 3)		**0.28**	**0.71**
Fat		**0.04**	**0.44**
Muscle		**0.10**	**0.11**
Liver		**0.46**	**0.87**

Extraction and hydrolysis of residues

Eggs and tissue samples were subjected to extraction and/or hydrolysis procedures for residue characterization and identification. The registrants provided adequate descriptions of the fractionation schemes used for the analysis of acephate residue in eggs and tissues. A flow chart of the extraction scheme was provided. During the extraction and fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction and fractionation procedures for eggs and tissues are summarized below.

Subsamples of hen matrices were extracted three times with ACN, and centrifuged. The resulting supernatants for each matrix were pooled. The remaining solids were then extracted three times with ACN:water (50:50, v:v) and centrifuged; the supernatants were combined with the ACN extracts. The ACN extracts were partitioned with hexane (hexane 1 extract) and the phases were allowed to separate. Any organic solvent remaining in the aqueous phase was removed by evaporation and the aqueous phase was partitioned two more times with hexane. The aqueous phase was reserved for HPLC analysis; in addition, the extract was lyophilized and redissolved in methanol for TLC analysis. The hexane phases were combined (hexane 2 extract). The nonextractable residues were extracted three times with ethyl acetate and centrifuged. The ethyl acetate extracts were combined.

The distribution of total radioactive residues in the extracts of [S-methyl-^{14}C]acephate- and [carbonyl-^{14}C]acephate-dosed hen matrices are presented in Tables 24 and 25, respectively.

Characterization and identification of ^{14}C-residues

Extracts and hydrolysates were analyzed by HPLC and TLC. The following HPLC methods were used: Method 1, a C-18 column and a gradient mobile phase of methanol, water, ACN, and 0.5 mM heptatriethyl ammonium phosphate in 1 mM potassium dihydrogen phosphate; Method 1-A, a C-18 column and a gradient mobile phase of methanol, ACN, and 0.5 mM heptatriethyl ammonium phosphate in 1 mM potassium dihydrogen phosphate; Method 2, a C-18 column and a gradient mobile phase of water and ACN; Method 3, a Nucleosil 5N$(CH_3)_2$ column and a gradient mobile phase of ACN and 0.01 M ammonium chloride (pH 7.0); Method 4, an Aminex ion-exclusion column and an isocratic mobile phase of 0.005 M sulfuric acid; Method 5, a Chrompack lipids column and a gradient mobile phase of ACN and dichloromethane; Method 6, a C-18 column and a gradient mobile phase of ACN and 0.5 mM heptatriethyl ammonium phosphate in 1 mM potassium dihydrogen phosphate; Method 7, a C-18 column and a gradient mobile phase of tetrahydrofuran, 0.1 aqueous phosphoric acid, and ACN; Method 8, a C-18 column and a gradient mobile phase of ACN and 0.05 trifluoroacetic acid; and Method 11, a C-18 column and a gradient mobile phase of ACN and 0.01 M ammonium acetate buffer. Nonlabeled standards were detected by UV (210, 215, 230, or 254 nm), and radioactivity was detected using a radioisotope detector. Metabolites were identified by comparison of retention times with those of the following reference standards: acephate, methamidophos, acetamide, acetic acid, SMPT, SMPAA, OMPT, OMAPAA, DMPT, and DMPT-CH_3, as well as palmitic acid, oleic acid, and N-acetyl-L-glutamic acid. Methods 1, 1-A, and 2 were used for analysis of the aqueous extracts of eggs and tissues; Method 3 was used for the confirmation of metabolites SMPT, DMPT, and SMPAA; Methods 4 and 6 were used for the confirmation of acetamide; Methods 5 and 7 were used for the analysis of fatty acids; and Methods 8 and 11 were used for the analysis of amino acids.

TLC analyses were conducted on silica gel (60C F-254) plates using the following solvent systems: Method 1, chloroform:methanol (9:1, v:v); or Method 2, ethanol:water (95:5, v:v). Nonlabeled standards were either visualized by spraying with 1 ammonium molybdate and 1 stannous chloride in 10 HCl or visualized using iodine vapor. Radioactivity was detected and quantitated using a linear analyzer.

The identification of acephate in all aqueous extracts by HPLC was confirmed by TLC analyses. In addition, acephate was isolated from the aqueous extracts of egg whites (both labels) and was identified by coinjection with the acephate standard using HPLC and TLC.

The identification of acetamide was confirmed by isolating acetamide from a sample of [carbonyl-^{14}C]acephate-treated liver and coinjecting the isolated metabolite with a [^{14}C]acetamide standard on HPLC.

The identification of metabolites SMPT, DMPT, and SMPAA was confirmed by isolating these metabolites from a sample of [S-methyl-^{14}C]acephate-treated liver. The isolated metabolites were analyzed by reverse-phase and normal-phase HPLC and then the metabolites were methylated using diazomethane and the HPLC analyses were repeated to confirm the identification of SMPT, DMPT, and SMPAA. In addition, the presence of SMPT in the aqueous extracts of egg white (S-methyl label) and fat (carbonyl label) and the presence of SMPAA in the aqueous extracts of all matrices except fat (S-methyl label only) was confirmed by TLC analyses.

The registrant isolated the unknown P1 from egg white and collected its UV spectrum using a diode array detector. The registrant stated that the UV spectrum and its HPLC peak shape suggested that unknown P1 was a polar natural product and was not a phosphate-containing molecule. The registrant noted that because unknown P1 eluted at the retention time of acephate in the HPLC analyses used for tissue extracts, P1 may have been present in tissues; however, it would be expected to be present in minor amounts. The registrant stated that unknown P2 was not further analyzed because it was present at low levels (<0.05 ppm).

To characterize/identify the radioactivity in the ethyl acetate extracts, the registrant saponified the ethyl acetate extract of egg yolks (carbonyl label; this extract contained the highest level of radioactivity of all ethyl acetate extracts) in aqueous potassium hydroxide and ethanol at reflux overnight. The solution was then extracted with ether, and the aqueous phase was acidified (pH <2) with concentrated HCl and extracted again with ether. The ether layer was isolated, washed with saturated sodium chloride solution, and evaporated to dryness to isolate the fatty acids. The fatty acid fraction contained the majority of the radioactivity (94.6 of radioactivity in extract). The registrant stated that the saponification procedure was vigorous enough to cleave carboxylic acid ester and phosphate ester groups. HPLC analysis indicated that the major radioactive peak coeluted with the standards of palmitic and oleic acid. The p-bromophenacyl esters of the isolated fatty acids were prepared and analyzed by GC/MS which confirmed the presence of palmitic and oleic acids. No quantitative data for the individual fatty acids were presented. Based on these analyses, the registrant attributed the radioactivity in the ethyl extracts of all hen matrices to fatty acids.

The registrant stated that TLC analyses of the combined hexane extracts (hexane 1 and hexane 2) of fat and liver samples (carbonyl label only) indicated the presence of nonpolar material, suggesting that residues were lipophilic.

To demonstrate the incorporation of radioactivity into amino acids, the registrant acid hydrolyzed the nonextractable residues of egg white (S-methyl label) in 6 N HCl (at reflux overnight) and evaporated the hydrolysate to dryness. The residues were redissolved in 0.05 trifluoroacetic acid buffer and analyzed by HPLC which resolved two areas of radioactivity. The major fraction was isolated, acetylated (using pyridine and acetic acid at room temperature for 2 hours), and

analyzed by HPLC, which resolved four major peaks of radioactivity. The largest peak was isolated, purified by HPLC, and identified as N-acetyl glutamic acid by MS and NMR. No quantitative data were presented.

Nonextractable residues (egg yolk, egg white, muscle, and liver samples only) were separately subjected to surfactant extraction (5 lauryl sulfate containing 25 mM DL-dithiothreitol, 38 C, 3-4 hours), dilute acid/base extraction (using 1 N HCl and then sodium hydroxide at room temperature), and enzyme hydrolysis (treatment with protease at 40 C overnight). For egg yolk and liver samples (both labels), the resulting supernatants were analyzed by HPLC and TLC as follows: for surfactant and enzyme hydrolysis supernatants, the supernatant was mixed with ACN and centrifuged, and the soluble portion was evaporated to remove ACN for HPLC analysis; for base hydrolysis supernatants, the supernatant was neutralized with 1 N HCl and filtered for HPLC analysis; and the supernatants were evaporated, the resulting aqueous residues lyophilized, and the dried residues dissolved in ACN for TLC analysis.

Summaries of the characterized and identified residues found in hen matrices following dosing with [S-methyl-^{14}C]acephate and [carbonyl-^{14}C]acephate are presented in Tables 26 and 27, respectively.

Table 24. Distribution of total radioactive residues in eggs and tissues from hens orally dosed with [S-methyl-^{14}C]acephate at 10 ppm in the diet (~5x the maximum theoretical dietary burden) for 3 days.

Fraction	% TRR	ppm	Characterization/Identification [a]
Egg whites (Day 3; TRR = 0.34 ppm)			
Aqueous	83.0	0.28	HPLC analysis resolved: Acephate 42.4% TRR 0.144 ppm Methamidophos 9.5% TRR 0.032 ppm SMPT 3.5% TRR 0.012 ppm DMPT 1.7% TRR 0.006 ppm SMPAA 1.7% TRR 0.006 ppm Unknown (P1) 17.9% TRR 0.061 ppm
Hexane 1	0.0	0.00	Not further analyzed (N/A).
Hexane 2	0.0	0.00	N/A.
Ethyl acetate	0.1	<0.001	N/A.
Nonextractable	28.3	0.096	A subsample was hydrolyzed (6 N HCl), acetylated, and subjected to HPLC purification procedures. The largest HPLC peak in the acetylated hydrolysate was identified as N-acetyl glutamic acid by MS and NMR. No quantitative data were presented. In addition, subsamples of nonextractable residues were separately subjected to surfactant extraction, dilute acid hydrolysis, dilute base hydrolysis, and protease digestion. The extraction/hydrolyses released radioactivity as follows: Surfactant extraction 0.82% TRR 0.003 ppm Dilute acid hydrolysis 1.8% TRR 0.006 ppm Dilute base hydrolysis 3.2% TRR 0.011 ppm Protease digestion 27.6% TRR 0.094 ppm No further analyses were conducted.
Egg yolks (Day 3; TRR = 0.17 ppm)			
Aqueous	47.5	0.081	HPLC analysis resolved: Acephate 32.6% TRR 0.056 ppm SMPT 3.3% TRR 0.006 ppm DMPT 0.9% TRR 0.002 ppm SMPAA 0.7% TRR 0.001 ppm
Hexane 1	1.7	0.003	N/A.
Hexane 2	1.7	0.003	N/A.
Ethyl acetate	11.8	0.020	N/A.
Nonextractable	40.6	0.069	Subsamples of nonextractable residues were separately subjected to surfactant extraction, dilute acid hydrolysis, dilute base hydrolysis, and protease digestion. The extraction/hydrolyses released radioactivity as follows: Surfactant extraction 16.3% TRR 0.028 ppm (Remaining solids 11.6% TRR 0.020 ppm)

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification [a]
			Dilute acid hydrolysis 1.6% TRR 0.003 ppm Dilute base hydrolysis 24.8% TRR 0.042 ppm (Remaining solids 23.2% TRR 0.040 ppm) Protease digestion 16.8% TRR 0.029 ppm (Remaining solids 17.4% TRR 0.030 ppm) No further analyses were conducted.
Fat (TRR = 0.04 ppm)			
Aqueous	63.2	0.025	HPLC analysis resolved: Acephate 25.7% TRR 0.010 ppm Methamidophos 2.7% TRR 0.001 ppm SMPT 17.0% TRR 0.007 ppm DMPT 1.1% TRR <0.001 ppm SMPAA 2.9% TRR 0.001 ppm
Hexane 1	0.3	<0.001	N/A.
Hexane 2	0.7	<0.001	N/A.
Ethyl acetate	10.4	0.004	N/A.
Nonextractable	42.4	0.017	N/A.
Muscle (TRR = 0.10 ppm)			
Aqueous	79.4	0.079	HPLC analysis resolved: Acephate 9.9% TRR 0.010 ppm Methamidophos 13.6% TRR 0.013 ppm
Hexane 1	0.5	<0.001	N/A.
Hexane 2	0.0	0.00	N/A.
Nonextractable	27.5	0.028	N/A.
Liver (TRR = 0.46 ppm)			
Aqueous	41.9	0.19	HPLC analysis resolved: Acephate 11.0% TRR 0.051 ppm SMPT 11.7% TRR 0.054 ppm DMPT 0.9% TRR 0.004 ppm SMPAA 5.9% TRR 0.027 ppm Unknown (P2) 9.3% TRR 0.043 ppm
Hexane 1	2.2	0.010	N/A.
Hexane 2	1.1	0.005	N/A.
Ethyl acetate	6.5	0.030	N/A.
Nonextractable	49.5	0.23	Subsamples of nonextractable residues were separately subjected to surfactant extraction, dilute acid hydrolysis, dilute base hydrolysis, and protease digestion.
Surfactant extraction	18.2	0.084	Majority of radioactivity precipitated when this fraction was mixed with ACN, indicating residues were not small molecules such as organophosphates. TLC analysis of ACN-soluble residues resolved two broad peaks; however, radioactivity levels were too low (0.01 ppm) for further analysis.

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification [a]
Remaining solids	2.1	0.010	N/A.
Dilute acid hydrolysis	5.0	0.023	N/A. The solids remaining were subjected to dilute base hydrolysis.
Dilute base hydrolysis	30.7	0.14	HPLC analysis resolved one major peak, which the registrant attributed to solubilized protein, and two minor peaks. When this fraction was mixed with ACN, all radioactive residues precipitated.
Remaining solids	28.0	0.13	N/A.
Protease digestion	44.5	0.20	The majority of radioactivity remained in solution when this fraction was mixed with ACN, indicating that radioactivity was associated with amino acids. TLC analysis showed broad areas of radioactivity indicating that residues were complex.
Remaining solids	8.6	0.040	N/A.

[a] Identification of acephate and metabolites other than methamidophos was confirmed by TLC analyses.

(continued; footnotes follow)

Table 25. Distribution of total radioactive residues in eggs and tissues from hens orally dosed with [carbonyl-^{14}C]acephate at 10 ppm in the diet (~5x the maximum theoretical dietary burden) for 3 days.

Fraction	% TRR	ppm	Characterization/Identification [a]
Egg whites (Day 3; TRR = 0.31 ppm)			
Aqueous	81.2	0.25	HPLC analysis resolved: Acephate 61.7% TRR 0.190 ppm Acetamide 10.5% TRR 0.032 ppm SMPT 4.2% TRR 0.013 ppm
Hexane 1	0.3	0.001	Not further analyzed (N/A).
Hexane 2	0.2	0.001	N/A.
Ethyl acetate	0.4	0.001	N/A.
Nonextractable	29.0	0.490	Subsamples of nonextractable residues were separately subjected to surfactant extraction, dilute acid hydrolysis, dilute base hydrolysis, and protease digestion. The extraction/hydrolyses released radioactivity as follows: Surfactant extraction 0.43% TRR 0.001 ppm Dilute acid hydrolysis 7.1% TRR 0.022 ppm Dilute base hydrolysis 5.6% TRR 0.017 ppm Protease digestion 27.1% TRR 0.084 ppm No further analyses were conducted.
Egg yolks (Day 3; TRR = 1.52 ppm)			
Aqueous	8.4	0.13	HPLC analysis resolved: Acephate 5.3% TRR 0.080 ppm Acetamide 1.0% TRR 0.016 ppm SMPT 0.9% TRR 0.014 ppm OMAPAA 0.2% TRR 0.003 ppm
Hexane 1	10.6	0.16	N/A.
Hexane 2	0.5	0.008	N/A.
Ethyl acetate	52.2	0.79	Saponified and extracted with ether; fatty acid fraction accounted for 49.4% TRR (0.75 ppm); HPLC isolated the major radioactive peak which coeluted with palmitic and oleic acids. HPLC and GC/MS analysis of the p-bromophenacyl derivatives of the isolated regions confirmed the incorporation of radioactivity into palmitic and oleic acid. No quantitative data were presented.
Nonextractable	19.9	0.30	Subsamples of nonextractable residues were separately subjected to surfactant extraction, dilute acid hydrolysis, dilute base hydrolysis, and protease digestion.
Surfactant extraction	8.0	0.12	Majority of radioactivity precipitated when this fraction was mixed with ACN, indicating residues were not small molecules such as organophosphates. HPLC analysis of ACN-soluble residues resolved two broad peaks; in TLC analysis no distinct component was observed.
Remaining solids	5.3	0.080	N/A.

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification [a]
Dilute acid hydrolysis	0.63	0.010	N/A. The solids remaining were subjected to dilute base hydrolysis.
Dilute base hydrolysis	9.8	0.15	HPLC analysis resolved one major peak, which the registrant attributed to solubilized protein, and several minor peaks. When this fraction was mixed with ACN, the majority of the radioactive residues precipitated.
Remaining solids	15.9	0.24	N/A.
Protease digestion	1.7	0.026	TLC analysis of ACN-soluble residues showed broad areas of radioactivity indicating that residues were complex.
Remaining solids	10.6	0.16	N/A.
Fat (TRR = 0.44 ppm)			
Aqueous	3.1	0.014	HPLC analysis resolved: Acephate 0.7% TRR 0.003 ppm Acetamide 0.6% TRR 0.003 ppm SMPT 0.7% TRR 0.003 ppm OMAPAA 0.3% TRR 0.001 ppm
Hexane 1	9.3	0.041	Combined with hexane 2 fraction.
Hexane 2	1.8	0.008	Combined with hexane 1 fraction; TLC analysis indicated that presence of nonpolar material, suggesting the residues were lipophilic.
Ethyl acetate	67.8	0.30	N/A.
Nonextractable	5.3	0.023	N/A.
Muscle (TRR = 0.11 ppm)			
Aqueous	55.2	0.061	HPLC analysis resolved: Acephate 40.8% TRR 0.045 ppm Acetamide 10.0% TRR 0.011 ppm SMPT 1.3% TRR 0.001 ppm OMAPAA 1.6% TRR 0.002 ppm
Hexane 1	4.8	0.005	N/A.
Hexane 2	4.3	0.005	N/A.
Ethyl acetate	5.1	0.006	N/A.
Nonextractable	43.0	0.047	Subsamples of nonextractable residues were separately subjected to surfactant extraction, dilute acid hydrolysis, dilute base hydrolysis, and protease digestion. The extraction/hydrolyses released radioactivity as follows: Surfactant extraction 3.7% TRR 0.004 ppm Dilute acid hydrolysis 1.9% TRR 0.002 ppm Dilute base hydrolysis 26.7% TRR 0.029 ppm Protease digestion 37.9% TRR 0.042 ppm No further analyses were conducted.
Liver (TRR = 0.87 ppm)			
Aqueous	20.3	0.18	HPLC analysis resolved:

(continued; footnotes follow)

Fraction	% TRR	ppm	Characterization/Identification [a]
			Acephate 2.4% TRR 0.021 ppm Acetamide 7.9% TRR 0.069 ppm SMPT 1.5% TRR 0.013 ppm OMAPAA 4.2% TRR 0.037 ppm
Hexane 1	18.0	0.16	Combined with hexane 2 fraction.
Hexane 2	6.8	0.059	Combined with hexane 1 fraction; TLC analysis indicated that presence of nonpolar material, suggesting the residues were lipophilic.
Ethyl acetate	22.5	0.20	N/A.
Nonextractable	34.4	0.30	Subsamples of nonextractable residues were separately subjected to surfactant extraction, dilute acid hydrolysis, dilute base hydrolysis, and protease digestion.
Surfactant extraction	8.7	0.076	Majority of radioactivity precipitated when this fraction was mixed with ACN, indicating residues were not small molecules such as organophosphates. In TLC analysis, no distinct component was observed.
Remaining solids	4.6	0.040	N/A.
Dilute acid hydrolysis	1.9	0.016	N/A. The solids remaining were subjected to dilute base hydrolysis.
Dilute base hydrolysis	13.4	0.12	HPLC analysis resolved two major peaks, which the registrant attributed to solubilized protein. When this fraction was mixed with ACN, the majority of the radioactive residues precipitated.
Remaining solids	24.1	0.21	N/A.
Protease digestion	24.8	0.22	The majority of radioactivity remained in solution when this fraction was mixed with ACN, indicating that radioactivity was associated with amino acids. TLC analysis showed broad areas of radioactivity indicating that residues were complex.
Remaining solids	10.3	0.090	N/A.

[a] Identification of acephate and metabolites other than OMAPAA was confirmed by TLC analyses.

(continued; footnotes follow)

Table 26. Summary of radioactive residues characterized/identified in eggs and tissues from laying hens orally dosed with [S-methyl-^{14}C]acephate at 10 ppm (~5x the maximum theoretical dietary burden) in the diet for 3 days.

	Egg white (TRR = 0.34 ppm)		Egg yolk (TRR = 0.17 ppm)		Fat (TRR = 0.04 ppm)		Muscle (TRR = 0.10 ppm)		Liver (TRR = 0.46 ppm)	
Fraction	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**
Identified [a]										
Acephate	42.4	0.144	32.6	0.056	25.7	0.010	9.9	0.010	11.0	0.051
Methamidophos	9.5	0.032	--	--	2.7	0.001	13.6	0.013	--	--
SMPT	3.5	0.012	3.3	0.006	17.0	0.007	--	--	11.7	0.054
DMPT	1.7	0.006	0.9	0.002	1.1	<0.001	--	--	0.9	0.004
SMPAA	1.7	0.006	0.7	0.001	2.9	0.001	--	--	5.9	0.027
Total identified	58.8	0.200	37.5	0.064	49.4	0.020	23.5	0.023	29.5	0.136
Characterized										
Unknown (P1)	17.9	0.061	--	--	--	--	--	--	--	--
Unknown (P2)	--	--	--	--	--	--	--	--	9.3	0.043
Hexane [b]	--	--	3.4	0.006	1.0	<0.001	0.5	<0.001	3.3	0.015
Ethyl acetate	0.1	<0.001	11.8	0.020	10.4	0.004	--	--	6.5	0.030
Protease hydrolysate [c]	27.6 [d]	0.094	16.8	0.029	--	--	--	--	44.5	0.20
Total identified/ characterized	104.4	0.35	69.5	0.12	60.8	0.024	24.0	0.024	63.6	0.29
Nonextractable	N/R [e]	N/R	17.4	0.030	42.4	0.017	27.5	0.028	8.6	0.040

[a] **See Figure 1 for chemical structures of identified metabolites.**

[b] **Combined hexane 1 and hexane 2 fractions.**

[c] **Nonextractable residues were separately subjected to surfactant, dilute acid/base hydrolysis, and protease digestion. Because protease digestion released the greatest amount of radioactivity (in general), the results of protease treatment are reported here.**

[d] **A subsample of nonextractable residues prior to protease digestion were subjected to strong acid hydrolysis, acetylation, and HPLC, MS, and NMR analyses, which confirmed the presence of [^{14}C]N-acetyl glutamic acid.**

[e] **N/R = not reported.**

Table 27. Summary of radioactive residues characterized/identified in eggs and tissues from laying hens orally dosed with [carbonyl-^{14}C]acephate at 10 ppm (~5x the maximum theoretical dietary burden) in the diet for 3 days.

	Egg white (TRR = 0.31 ppm)		Egg yolk (TRR = 1.52 ppm)		Fat (TRR = 0.44 ppm)		Muscle (TRR = 0.11 ppm)		Liver (TRR = 0.87 ppm)	
Fraction	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**	**% TRR**	**ppm**
Identified [a]										
Acephate	61.7	0.190	5.3	0.080	0.7	0.003	40.8	0.045	2.4	0.021
Acetamide	10.5	0.032	1.0	0.016	0.6	0.003	10.0	0.011	7.9	0.069
SMPT	4.2	0.013	0.9	0.014	0.7	0.003	1.3	0.001	1.5	0.013
OMAPAA	--	--	0.2	0.003	0.3	0.001	1.6	0.002	4.2	0.037
Total identified	76.4	0.24	7.4	0.11	2.3	0.010	53.7	0.059	16.0	0.14
Characterized										
Hexane [b]	0.5	0.002	11.1	0.17	11.1	0.049	9.1	0.010	24.8	0.22
Ethyl acetate	0.4	0.001	52.2 [c]	0.79	67.8	0.30	5.1	0.006	22.5	0.20
Protease hydrolysate [d]	27.1	0.084	1.7	0.026	--	--	37.9	0.042	24.8	0.22
Total identified/ characterized	104.4	0.32	72.4	1.10	81.2	0.36	105.8	0.12	88.1	0.77
Nonextractable	N/R [e]	N/R	10.6	0.16	5.3	0.023	N/R	N/R	10.3	0.090

[a] **See Figure 1 for chemical structures of identified metabolites.**
[b] **Combined hexane 1 and hexane 2 fractions.**
[c] **The majority of radioactive residues in this fraction were saponifiable (49.4% TRR, 0.75 ppm). HPLC and GC/MS analysis confirmed the incorporation of radioactivity into palmitic and oleic acids; however, no quantitative data were presented.**
[d] **Nonextractable residues were separately subjected to surfactant, dilute acid/base hydrolysis, and protease digestion. Because protease digestion released the greatest amount of radioactivity (in general), the results of protease treatment are reported here.**
[e] **N/R = not reported.**

CBRS notes that the data presented here are from an amended report. When the aqueous extracts of S-methyl-treated muscle were originally analyzed by HPLC (using Method 1), acephate was found to be the major residue (63.6 TRR, 0.062 ppm) and low levels of methamidophos (0.003 ppm), DMPT (0.001 ppm), and SMPAA (0.004 ppm) were detected (reported in MRID 43971605). When radiovalidation of the enforcement method (discussed below) was conducted, the levels of acephate found using the GC/FPD enforcement method were much lower than 0.062 ppm. It was then determined that in the original HPLC analysis, acephate metabolites eluted at the same retention time as acephate, and so HPLC analyses of the muscle extract were repeated using a different mobile phase (HPLC Method 1-A). The results reported in Tables 24 and 26 are those of the repeat analyses.

Storage stability

All samples were stored frozen (<-15 C) until extraction and analysis. HPLC analyses of sample extracts were completed within 35 days of sample collection except for the repeat analyses of aqueous extract of muscle (S-methyl label only), which were conducted within ~15-16 months of sample collection. The additional characterization/identification of radioactivity as fatty acids, amino acids, acetamide, and polar metabolites was conducted within 80-159 days (~3-5 months) of sample collection. The registrant stated that supporting storage stability data were not required for this study because samples were analyzed within 1 month of collection.

The registrant must submit data demonstrating that the metabolic profile of radioactive residues in muscle did not change significantly between the initial analysis (completed within 35 days of collection) and the repeat analyses (conducted ~15 months later).

Study summary

The qualitative nature of the residue in poultry is adequately understood pending submission of additional storage stability data. Following oral administration of [S-methyl-^{14}C]acephate or [carbonyl-^{14}C]acephate to laying hens for 3 days at 10 ppm (~5x the maximum dietary burden), the TRR, respectively, were 0.08-0.34 ppm and 0.02-0.31 ppm in egg white, 0.02-0.17 ppm and <0.021-1.52 ppm in egg yolk, 0.04 ppm and 0.44 ppm in fat, 0.10 ppm and 0.11 ppm in muscle, and 0.46 ppm and 0.87 ppm in liver.

The majority of ^{14}C-residues, ~61->100 , were characterized/identified in egg white, egg yolk, fat, and liver of hens following dosing with the S-methyl-labeled test substance; only 24 of ^{14}C-residues in muscle were characterized/identified. Acephate was identified in egg white (42.4 TRR, 0.144 ppm), egg yolk (32.6 TRR, 0.056 ppm), fat (25.7 TRR, 0.010 ppm), muscle (9.9 TRR, 0.010 ppm), and liver (11.0 TRR, 0.051 ppm). Methamidophos was identified in egg white (9.5 TRR, 0.032 ppm), fat (2.7 TRR, 0.001 ppm), and muscle (13.6 TRR, 0.013

ppm). Other identified metabolites were SMPT (3.3-17.0 TRR, 0.006-0.054 ppm), DMPT (0.9-1.7 TRR, <0.001-0.006 ppm), and SMPAA (0.7-5.9 TRR, 0.001-0.027 ppm); these metabolites were each detected in egg white, egg yolk, fat, and liver. The registrant demonstrated the incorporation of radioactivity into glutamic acid in egg white. One unknown (P1) which detected in egg white at 17.9 TRR (0.061 ppm) was characterized to be a polar natural product based on its HPLC peak shape and UV spectrum. Another unknown, detected in liver at 9.3 TRR (0.043 ppm), was not identified because of its low residue level.

The majority of ^{14}C-residues, ~72->100 , were characterized/identified in egg white, egg yolk, fat, muscle, and liver following dosing with the carbonyl-labeled test substance. Acephate was identified in egg white (61.7 TRR, 0.190 ppm), egg yolk (5.3 TRR, 0.080 ppm), fat (0.7 TRR, 0.003 ppm), muscle (40.8 TRR, 0.045 ppm), and liver (2.4 TRR, 0.021 ppm). Acetamide was identified in egg white (10.5 TRR, 0.032 ppm), egg yolk (1.0 TRR, 0.016 ppm), fat (0.6 TRR, 0.003 ppm), muscle (10.0 TRR, 0.011 ppm), and liver (7.9 TRR, 0.069 ppm). Other identified metabolites were SMPT (all matrices, 0.7-4.2 TRR, 0.001-0.014 ppm) and OMAPAA (all matrices except egg white, 0.2-4.2 TRR, 0.001-0.037 ppm). The registrant demonstrated the incorporation of radioactivity into fatty acids (palmitic and oleic acid) in egg yolks; 49.4 of egg yolk TRR (0.75 ppm) was saponifiable.

Proposed metabolic pathway in animals

Based on the findings of the metabolism studies, the registrant has proposed a metabolic pathway for acephate in animals. The registrant concluded that acephate undergoes enzyme hydrolysis, oxidation, and incorporation of intermediates into the biosynthetic carbon pools. Hydrolysis of the P-N or C-N bond would form acetamide and DMPT or methamidophos and an enzyme-acetyl complex. The acetamide could then be deaminated for incorporation into naturally occurring acetamide, amino acids and proteins, and fatty acids and lipids. The registrant stated that the S-methyl label was preferentially incorporated into amino acids and proteins. This could occur via formation of OMPT, or via hydrolysis of the P-S bond to form OMAPAA. In addition, hydrolysis of the P-O bond yields SMPT which can be further degraded to SMPAA and an acetyl group. The registrant noted that hydrolysis of acephate ultimately leads to phosphoric acid.

HED has no concerns about the metabolites, SMPAA, SMPT, DMPT, and OMAPAA. These metabolites are not cholinesterase inhibitors and it has been concluded that theeffects of cholinesterase inhibition would far outweigh the toxicological significance of other effects. [Consultation with Alberto Protzel, Toxicology Branch II, 4/3/97] The residues of concern are therefore the parent, acephate and its cholinesterase inhibiting metabolite, methamidiphos.

Radiovalidation of the enforcement method

Valent has submitted radiovalidation data (1996; MRIDs 43971608 and 44037804) for a GC/flame photometric detection (FPD) method, Valent method RM-12A-9. Radiovalidation was conducted by Valent (Dublin, CA) using radiolabeled samples from the goat and hen metabolism studies.

Samples of homogenized milk (day 2) and liver from the [S-methyl-^{14}C]acephate goat metabolism study and egg white (day 3) and muscle from the [S-methyl-^{14}C]acephate hen metabolism study were utilized. Samples of egg white and muscle were mixed with phosphoric acid and water, and samples of liver were mixed with water prior to extraction. The samples were then blended with ethyl acetate and sodium sulfate and filtered. The ethyl acetate extraction and filtration were repeated twice more. The resulting filtrates were combined and evaporated to dryness using a rotary evaporator. The dried residue was then redissolved in ether, transferred to a silica gel column, and eluted with ether followed by 5 methanol in ether; the resulting eluate was evaporated to dryness, dissolved in acetone, and applied to a GC equipped with a 50 phenyl-methyl silicone column or a dimethylpolysiloxane column and an FPD. The reported LODs were 0.02 ppm and 0.01 ppm for acephate and methamidophos, respectively, and the reported LOQs were 0.05 ppm and 0.02 ppm, respectively.

Sample calculations, supporting data, and representative chromatograms depicting GC analysis of acephate and methamidophos were provided. The radiovalidation data comparing the results of the GC/FPD analysis to data obtained from the goat and hen metabolism studies are presented in Table 28. Concurrent method recoveries from samples of untreated commodities fortified with acephate and methamidophos are also presented in Table 28.

Table 28. Determination of acephate and methamidophos in samples of milk and liver from S-methyl-dosed goats, and egg white and muscle from S-methyl-dosed hens from the metabolism studies using HPLC radiochemical analysis and the GC/FPD enforcement method.

Commodity	Method	Acephate, ppm	Methamidophos, ppm
Milk	HPLC Radiochemical Analysis	0.077	0.004 [a]
	Method RM-12A-9	0.068	0.002
Liver	HPLC Radiochemical Analysis	0.046	<0.01
	Method RM-12A-9	0.042	0.003
Egg White	HPLC Radiochemical Analysis	0.144	0.025 [b]
	Method RM-12A-9	0.119	0.009
Muscle	HPLC Radiochemical Analysis	0.010	0.010 [c]

	Method RM-12A-9	**0.010, 0.015**	**0.002, 0.003**
Concurrent Method Recovery [d]			
Milk	**Method RM-12A-9**	**94.7, 104**	**70.4, 72.4**
Liver	**Method RM-12A-9**	**94.5, 96.5**	**91.8, 96.6**
Egg White	**Method RM-12A-9**	**70.0, 78.7**	**53.4, 61.9**
Muscle	**Method RM-12A-9**	**89.3, 100, 111**	**95.7, 101, 104**

[a] **Equivalent to 0.005 ppm acephate equivalents.**
[b] **Equivalent to 0.032 ppm acephate equivalents.**
[c] **Equivalent to 0.013 ppm acephate equivalents.**
[d] **Samples of milk, liver, and muscle were fortified with acephate and methamidophos at 0.05 and 0.02 ppm, respectively, and samples of egg white were fortified at 0.50 and 0.20 ppm, respectively.**

The submitted radiovalidation data for enforcement method RM-12A-9 are adequate to satisfy radiovalidation data requirements. The method adequately recovered residues of acephate from samples of milk, goat liver, egg white, and muscle from goats and hens treated with [S-methyl-^{14}C]acephate (from the metabolism studies). Although the recovery of methamidophos from these same samples was low, levels of methamidophos were near to or below the method LOQ in the samples.

Residue Analytical Methods

Samples from the submitted field trial studies with mint hay and processing study with peanut processed commodities were analyzed for residues of acephate and its metabolite methamidophos using GC Method RM-12A-6. Sample analyses were performed by Valent Technical Center (Dublin, CA). The LODs for each matrix were 0.02 ppm for acephate and 0.01 ppm for methamidophos.

Briefly, samples were extracted with ethyl acetate, acetonitrile/hexane, or acetonitrile. Samples were cleaned up by silica gel column chromatography. Additional cleanups using acetonitrile/hexane partitioning or a second silica gel column were occasionally required. Residues were quantitated by GC using a flame photometric detector (FPD) in the phosphorous mode, and either a HP-17 (50 phenyl:50 methylpolysiloxane) or DB-1 (dimethylpolysiloxane) column. Sample calculations and chromatograms were submitted.

Concurrent recovery data were submitted for mint hay and peanut commodities. The results of concurrent method analyses of fortified untreated samples are presented in Table 29. These data indicate that Method RM-12A-6 is adequate for determining residues of acephate and its metabolite methamidophos in/on mint hay, and peanuts and its processed commodities.

Table 29. Concurrent method recoveries of acephate and methamidophos from fortified untreated samples of commodities from the submitted field trial and processing studies.

Crop	Commodity	Acephate		Methamidophos	
		Fortification Levels, ppm	% Recovery [a]	Fortification Levels, ppm	% Recovery [a]
Mint	hay	0.05-2.0	75-114 (14); 122, 127	0.02-0.80	68; 70-110 (15)
Peanuts	nutmeat	0.05, 0.25	71-109 (6)	0.02, 0.10	62-68 (4); 83, 93
	hulls	0.05-1.0	93-109 (4)	0.02-0.40	78-99 (4)
	expeller presscake	0.05, 0.25	117; 138	0.02, 0.10	95, 111
	solvent-extracted presscake	0.05, 0.25	101, 116	0.02, 0.10	72, 86
	expeller crude oil	0.25	124, 130	0.10	94, 106
	solvent-extracted crude oil	0.05, 0.25	102; 125	0.02, 0.10	79, 107
	refined oil	0.05, 0.25	101, 107	0.02, 0.10	95, 97
	bleached oil	0.05, 0.25	72, 112	0.02, 0.10	55; 100
	deodorized oil	0.05, 0.25	96, 106	0.02, 0.10	73, 83

[a] All values represent a single sample unless otherwise noted in parentheses; recovery values outside the acceptable 70-120% range are listed separately.

Storage Stability Data

Mint hay samples from the submitted field trial were dried outdoors for 1-6 days after cutting. After drying, hay samples were frozen and shipped via freezer truck to the analytical laboratory (Valent Technical Center, Dublin, CA) where the samples were stored frozen (-20 C) until maceration, extraction, and analysis. The total storage intervals between harvest and analysis were 42-106 days (~1-3 months) for mint hay.

The peanut samples from the field of the processing study were dug up and field dried for 13 days. After drying, peanuts were shelled and the nutmeats and hulls were packaged separately and frozen. Shelled peanut samples were shipped by freezer truck to the analytical laboratory (Valent Technical Center, Dublin, CA). Whole unshelled peanuts were also collected after field drying, stored frozen, and then shipped via freezer truck to Texas A & M University (Bryan, TX) for processing. Whole peanuts were stored frozen at Texas A & M until processing. Once processed, samples were stored frozen until overnight delivery to the analytical laboratory (Valent Technical Center, Dublin, CA). All samples were stored frozen (-20 C) at the analytical laboratory until maceration, extraction, and analysis. The total storage intervals between harvest and analysis were 105-217 days (~3-7 months) for peanut nutmeat, 78-181 days (~3-6 months) for peanut hulls, 77-79 days (~3 months) for peanut presscake, and 57-87 days (~2-3 months) for peanut oil (crude, refined, bleached, and deodorized).

The registrant references previously submitted storage stability data for residues of acephate in/on mint hay (MRID 41081601), and peanut nutmeat and hulls (MRID 44025201). These studies are currently under review by CBRS. The registrant stated that the cited studies indicate that residues of acephate and methamidophos are stable in/on macerated nutmeat stored frozen for up to 14 months; in/on peanut hulls stored frozen for up to 10 months; and in/on mint hay stored frozen for up to 12 months.

The registrant has submitted (1995; MRID 43971611) additional storage stability data for peanut oil in support of the peanut processing study. An untreated peanut oil sample was fortified with 0.25 ppm acephate and 0.10 ppm methamidophos. Duplicate samples were aliquotted and analyzed at 0, 74, and 130 days after frozen storage (~-20 C). Control and fortified samples were analyzed for residues of acephate and methamidophos using Method RM-12A-6. The LODs for peanut oil were 0.02 ppm for acephate and 0.01 ppm for methamidophos. The registrant states that storage stability of acephate and methamidophos in/on presscake can be assumed, since adequate storage stability data have been provided for peanut nutmeat and oil. The results of the storage stability study on peanut oil are presented in Table 30.

Table 30. Storage stability and concurrent method recovery (fresh fortification recovery) of acephate and its metabolite methamidophos in/on fortified samples of peanut oil stored frozen at -20 C.

Commodity	Storage Period, Months	Fresh Fortification Recovery, %	Storage Stability Recovery, %	Corrected Storage Stability Recovery, % [a]
Acephate				
Peanut, oil	**0**	**--**	**124, 130**	**--**
	2	**91**	**79, 94**	**87, 104**
	4	**100**	**95, 98**	**95, 98**
Methamidophos				
Peanut, oil	**0**	**--**	**94, 106**	**--**
	2	**75**	**67, 81**	**89, 107**
	4	**93**	**95, 97**	**103, 105**

[a] **Calculated by dividing the storage stability recovery by the average fresh fortification recovery.**

Study summary

The supplemental storage stability data are acceptable and may be used to partially satisfy reregistration requirements for storage stability data. The data indicate that fortified residues of acephate and its metabolite methamidophos are relatively stable under frozen storage conditions (-20 C) for at least 4 months in/on peanut oil.

A determination regarding the adequacy of available storage stability data to support the storage intervals and conditions of samples from the submitted mint hay field trial and peanut processing studies will be made when MRIDs 41081601 and 44055201 have been reviewed by CBRS.

Magnitude of the Residue in Plants

Mint, hay

Established tolerance: A tolerance of 15 ppm has been established for the combined residues of acephate and its cholinesterase-inhibiting metabolite methamidophos in/on mint hay, of which no more than 1 ppm is methamidophos [40 CFR §180.108(a)].

Use patterns registered to Valent: The 75 SC formulations (EPA Reg. Nos. 59639-26 and 59639-89, and EPA SLN No. OR890015) are registered for two foliar applications to mint (peppermint and spearmint) at 1.0 lb ai/A/application using ground or aerial equipment.

Applications should be made in a minimum of 20 gal/A of water when using ground equipment, and 5 gal/A of water when using aerial equipment. The established PHI is 14 days and the maximum seasonal rate is 2.0 lb ai/A. The use of spent mint hay (hay after oil is extracted) for feed for dairy animals is prohibited.

Discussion of the data: Valent has submitted data (1995; MRID 43971610) from three tests depicting residues of acephate and its metabolite methamidophos in/on fresh mint hay. Trials were conducted in OR(2) and WA. All applications were made using the 75 SC formulation (EPA Reg. No. 59639-26) at ~1.0 lb ai/A/application using aerial equipment. At each site, both of the following treatment programs were conducted: (i) first-growth mint was cut 14, 21, and 28 days following a single application, and second-growth mint was cut at three intervals coinciding with normal harvest times; and (ii) second-growth mint was cut 14, 21, and 28 days following one application to first-growth mint and a second application to second-growth mint. In the WA trial only, the following additional treatment programs were added: (i) first-growth mint was cut 14, 21, and 28 days following two applications, with a 28-day retreatment interval, and second-growth mint was cut at normal harvest; and (ii) second-growth mint was cut 14, 21, and 28 days following two applications to first-growth mint, with a 28-day retreatment interval, and a third application to second-growth mint. Applications were made in 9.3-10.2 gal/A of water.

One control and duplicate treated samples were collected by hand from each test. Hay samples were dried outdoors for 1-6 days. After drying, hay samples were frozen and shipped via freezer truck to the analytical laboratory (Valent Technical Center, Dublin, CA) where the samples were stored frozen (-20 C) until analysis.

Residues in/on treated and untreated mint hay were determined using a GC/FPD method (RM-12A-6). The LODs for acephate and methamidophos in all matrices were 0.02 and 0.01 ppm, respectively. The results of the mint hay field trials are presented in Table 31. Apparent residues of acephate and methamidophos were less than the LOD (<0.02 and <0.01 ppm) in/on fifteen samples of untreated mint hay. Detectable residues of acephate and/or its metabolite methamidophos were observed in/on three samples of untreated mint hay at 0.050-0.220 ppm acephate and <0.01-0.023 ppm methamidophos.

Table 31. Residues of acephate and methamidophos in/on mint hay cut/harvested at various intervals following different treatment programs with the 75% SC formulation.

Test Site	Treatment Program	PHI, days	Residues, ppm [a]	
			Acephate	Methamidophos
OR1	One application to first-growth mint at 1.0 lb ai/A; first- and second-growth hay collected.	14	1.9, 2.9	0.18, 0.29
		21	3.5, 3.6	0.40, 0.40
		28	0.53, 1.2	0.06, 0.13
		88	<0.02, 0.02	<0.01, <0.01
		95	<0.02, <0.02	<0.01, <0.01
		102	<0.02, <0.02	<0.01, <0.01
	One application to first-growth mint and one application to second-growth mint at 1.0 lb ai/A/application; second-growth hay collected.	14	5.8, 8.6	0.75, 0.98
		21	1.8, 2.1	0.27, 0.31
		28	0.70, 0.89	0.17, 0.19
OR2	One application to first-growth mint at 1.0 lb ai/A; first- and second-growth hay collected.	14	2.1, 2.8	0.25, 0.34
		21	1.0, 1.2	0.13, 0.13
		28	0.38, 0.42	0.05, 0.06
		88	0.02, 0.23 [b]	<0.01, 0.02 [b]
		95	<0.02, <0.02	<0.01, <0.01
		102	<0.02, <0.02	<0.01, <0.01
	One application to first-growth mint and one application to second-growth mint at 1.0 lb ai/A/application; second-growth hay collected.	14	11, 11	1.0, 1.1
		21	3.1, 3.9	0.36, 0.35
		28	1.1, 1.3	0.15, 0.17
WA	One application to first-growth mint at 1.0 lb ai/A; first- and second-growth hay collected.	14	1.5, 1.8	0.27, 0.31
		21	0.40, 0.45	0.09, 0.10
		28	0.06, 0.07	0.02, 0.03
		93	<0.02, 0.06	<0.01, 0.01
		100	<0.02, <0.02	<0.01, <0.01
		107	<0.02, <0.02	<0.01, <0.01
	One application to first-growth mint and one application to second-growth mint at 1.0 lb ai/A/application; second-growth hay collected.	14	4.1, 4.2	0.55, 0.54
		21	1.8, 1.9	0.32, 0.32
		28	0.41, 0.51	0.06, 0.11
WA	Two applications to first-growth mint at 1.0 lb ai/A/application; first- and	14	1.9, 2.0	0.33, 0.34
		21	0.45, 0.68	0.10, 0.12

(*continued; footnotes follow*)

Test Site	Treatment Program	PHI, days	Residues, ppm [a]	
			Acephate	Methamidophos
	second-growth hay collected.	28	0.09, 0.09	0.03, 0.03
		93	<0.02, 0.12	<0.01, 0.01
		100	<0.02, 0.06	<0.01, 0.01
		107	<0.02, <0.02	<0.01, <0.01
	Two applications to first-growth mint and one application to second-growth mint at 1.0 lb ai/A/application; second-growth hay collected.	14	4.1, 5.1	0.52, 0.68
		21	1.7, 1.8	0.28, 0.30
		28	0.51, 0.68	0.09, 0.07

[a] Residues in treated samples were not corrected for concurrent recovery.
[b] The highest residue value determined from triplicate analyses is reported.

Geographic representation of residue data is adequate. Field trials were conducted at the test sites specified in the Update (1/29/92).

Study summary

The submitted data indicate that residues of acephate and methamidophos will not exceed the established tolerances of 15 ppm (for combined residues of acephate and methamidophos) and 1 ppm (for methamidophos) in/on mint hay harvested 14 days following two applications of the 75 SC formulation at 1.0 lb ai/A/application (1x the maximum seasonal rate) using aerial equipment. Residues of acephate were 1.9-11 ppm and residues of methamidophos were 0.33-1.1 ppm in/on samples collected 14 days following one application to first-growth mint and one application to second-growth mint or following two applications to first-growth mint.

Previously submitted data (MRID 40508503; reviewed in the Update) for fresh mint hay indicated that the established tolerance for the combined residues of acephate and methamidophos was too low. Residues exceeded the tolerance in/on two samples for mint hay collected 14 days following a single application of the 75 SC formulation at 1 lb ai/A (0.5x the maximum seasonal rate) using aerial equipment; maximum residues of acephate were 26.2 ppm and maximum residues of methamidophos were 1.62 ppm. Based on these data, the Update required that the registrant modify the product labels to lengthen the PHI, reduce the maximum number of applications, or reduce the maximum single application rate, and submit supporting residue data. However, the current field trial data reflect the registered use pattern which has not been amended. The registrant must propose higher tolerances for residues of acephate and methamidophos in/on mint hay at 27 ppm and 2 ppm, respectively. Increased tolerances may

(*continued; footnotes follow*)

not be required if the registrant can provide an adequate explanation for the previously reported tolerance-exceeding residues.

According to Table 1 (OPPTS 860.100), the RAC of peppermint and spearmint is "tops (leaves and stems)"; the previous livestock feeds table listed "hay" to be the RAC for mint. The Agency considers any tolerances for "mint hay" to also cover residues from "fresh market mint" or "bunched mint"; see memo by G. J. Herndon (CBTS No. 10276, DP Barcode D180904, 12/10/92). Appropriate changes in the RAC definition for mint, as stated in 40 CFR §180.108(a), will be made at issuance of the Acephate RED.

Magnitude of the Residue in Processed Food/Feed

Peanuts

Established tolerances: Tolerances have been established for the combined residues of acephate and its cholinesterase-inhibiting metabolite methamidophos in/on peanuts at 0.2 ppm and in/on peanut hulls at 5 ppm [40 CFR §180.108(a)]. No feed additive tolerances are established for the processed commodities of peanuts.

Use patterns registered to Valent: The 75 SC formulations (EPA Reg. Nos. 59639-26 and 59639-89) are registered for multiple foliar applications to peanuts at 0.25-1.0 lb ai/A/application using ground or aerial equipment as necessary to maintain control of insect infestation. Applications should be made in a minimum of 10 gal/A of water when using ground equipment, and 5 gal/A of water when using aerial equipment. The established PHI is 14 days of digging. No maximum seasonal rate or maximum number of applications is established. The feeding/grazing of treated forage or hay to livestock is prohibited.

The 75 SC formulation is also registered for application as the following: (i) as a dry powder to peanut seed in hopper/planter boxes at 0.19 lb ai/100 lbs of seed; the use of treated seed for food/feed purposes or for processing for oil is prohibited (SLNs AL940001, FL940002, GA940001, GA960002, and VA930005); and (ii) a single in-furrow spray at planting to peanuts grown on 36-inch or greater row spacing at 0.75-1.0 lb ai/A in a minimum of 3 gal/A of water using ground equipment; the feeding/grazing of treated forage or hay to livestock is prohibited (NC930003 and VA920003).

The 15 G formulation is registered for an in-furrow application to peanuts at planting at 0.75-1.0 lb ai/A using ground equipment; the feeding/grazing of treated forage or hay to livestock is prohibited (NM930002 and TX950003). We note that the federal registered label for the 15 G (EPA Reg. No. 59639-75) does not have registered uses on peanuts.

(continued; footnotes follow)

Discussion of the data: Valent has submitted data (1995; MRID 43971611) depicting the potential for concentration of residues of acephate and its metabolite methamidophos in the processed commodities of peanuts. One trial with four plots (one untreated and three treated plots) was conducted in GA. Four foliar applications, with ~7-day retreatment intervals of the 75 SC formulation were made to peanuts at 1.0, 2.0 or 5.0 lb ai/A/application (1x, 2x, or 5x the maximum single application rate) in 38-39 gal/A of water using a tractor boom sprayer. Peanuts were dug up 14 days following the last application and left to field dry for 13 days. After drying, peanuts (for field samples) were shelled, and the nutmeat and hulls were frozen and shipped to the analytical laboratory (Valent Technical Center, Dublin, CA). Whole unshelled peanuts from the 5x trial were also collected after field drying, frozen, and shipped to Texas A & M University (Bryan, TX) for processing.

At the processing facility, treated and untreated whole peanut samples were processed into kernels (nutmeat), hulls (shells), presscake from the expeller, crude oil from the expeller, presscake after solvent extraction (meal), crude oil after solvent extraction, refined oil, bleached oil, and deodorized oil using a small scale processing procedure which simulated standard industrial processing conditions. Briefly, peanut samples were dried and then cleaned by aspiration and screening. The hulls were removed mechanically. Peanut nutmeats were heat-conditioned and pressed in an expeller to release crude oil. The resulting presscake was flaked and the residual crude oil was extracted using hexane. The solvent-extracted presscake (meal) was desolventized. The crude oil fractions obtained from the expeller and from solvent extraction were combined and refined to generate refined oil and soapstock. The refined oil was bleached and deodorized. The processed samples were frozen and shipped to the analytical laboratory (Valent Technical Center, Dublin, CA) for residue analysis.

Residues in/on treated and untreated peanuts and its processed commodities were determined using a GC/FPD method. The LODs for acephate and methamidophos in all matrices were 0.02 and 0.01 ppm, respectively. The results of the peanut processing study are presented in Table 32. Apparent residues of acephate and methamidophos were less than the LOD (<0.02 and <0.01 ppm) in/on three samples of untreated peanut nutmeat, two samples of untreated hulls, and one sample each of presscake from the expeller, crude oil from the expeller, presscake after solvent extraction (meal), crude oil after solvent extraction, refined oil, bleached oil, and deodorized oil processed from untreated peanuts.

Table 32. Residues of acephate and its metabolite methamidophos in the processed commodities of peanuts dug up 14 days (and field dried for 13 days) following four foliar applications of the 75% SC formulation at 5.0 lb ai/A/application (20 lb ai/A).

	Uncorrected Residues, ppm [a]	Concentration/Reduction Factor [b]

(*continued; footnotes follow*)

Substrate	Acephate	Methamidophos	Acephate	Methamidophos
Peanut, nutmeat (field)	**0.06**	**0.01**	**--**	**--**
Peanut, hulls (field)	**1.6**	**0.25**	**--**	**--**
Peanut, nutmeat (processor)	**0.09**	**0.02**	**--**	**--**
expeller presscake	**0.18**	**0.05**	**3x**	**5x**
solvent-extracted presscake	**0.12**	**0.03**	**2x**	**3x**
expeller crude oil	**<0.02**	**<0.01**	**<0.3x**	**<1x**
solvent-extracted crude oil	**<0.02**	**<0.01**	**<0.3x**	**<1x**
refined oil	**<0.02**	**<0.01**	**<0.3x**	**<1x**
bleached oil	**<0.02**	**<0.01**	**<0.3x**	**<1x**
deodorized oil	**<0.02**	**<0.01**	**<0.3x**	**<1x**
Peanut, hulls (processor)	**1.5**	**0.27**	**--**	**--**

[a] **The highest residue value of replicate analyses of a single sample is reported.**
[b] **Concentration factors were calculated using the peanut nutmeat samples from the field.**

Study summary

The submitted peanut processing data are adequate and indicate that residues of acephate and its metabolite methamidophos do not concentrate in refined oil processed from peanuts with detectable residues. However, the data indicate that residues of acephate and methamidophos may concentrate in peanut meal at 2x and 3x, respectively. The other processed commodities reported are not of concern; only meal (solvent-extracted presscake) and refined oil are considered significant processed commodities of peanuts (OPPTS 860.1000, Table 1).

Adequate peanut field trial data were presented in the Addendum to the Acephate Reregistration Standard dated 10/5/84. Combined residues of acephate and methamidophos were reported as 0.02-0.09 ppm (<0.01-0.02 ppm methamidophos) in/on peanuts dug 14-52 days after 1-4 applications of the 75 SC formulation at 0.5-2.0 lb ai/A/application. Assuming a worst-case scenario, since individual acephate residue levels were not reported, the HAFT would be 0.08 ppm acephate (maximum combined residues of 0.09 ppm minus 0.01 ppm, the minimum methamidophos residues) and 0.02 ppm methamidophos. Based on these HAFTs, the maximum expected acephate and methamidophos residues in peanut meal would be 0.16 and

(continued; footnotes follow)

0.06 ppm, respectively. Since the expected residues in peanut meal do not exceed the recommended individual tolerances for acephate and methamidophos for the RAC (0.2 and 0.1 ppm, respectively), no tolerances for peanut meal are required.

AGENCY MEMORANDA CITED IN THIS REVIEW

CBTS No.: 10276
DP Barcode: D180904
Subject: Request for Clarification of the Commodity Definition of Mint Hay.
From: G. J. Herndon
To: M. Mautz/R.Forrest
Dated: 12/10/92
MRID(s): None

MASTER RECORD IDENTIFICATION NUMBERS

References (used):

43971602 Baker, F.; Bautista, A.; Rose, J. (1996) A Metabolism Study with (S-(carbon 14)H3)- and (N-(carbon 14)(O)CH3)-Acephate in Lettuce: Lab Project Number: 471W: 4-194-0863: 94.225. Unpublished study prepared by PTRL West, Inc. 243 p.

43971603 Baker, F.; Bautista, A.; Rose, J. (1996) A Metabolism Study With (S-(carbon 14)H3)- and (N-(carbon 14)(O)CH3)-Acephate in Beans: Lab Project Number: 472W: 4-194-0864: 98895.Unpublished study prepared by PTRL West, Inc. 368 p.

43971604 Huhtanen, K.; Turck, P. (1996) Distribution and Metabolism of (carbon 14)Acephate in Lactating Goats: Lab Project Number: 94-0097: 6095-94-0097-EF-001: 6095-94-0097-EF-000. Unpublished study prepared by Department of Environmental and Metabolic Fate, Ricerca, Inc. 244 p.

43971606 Lai, J. (1996) Validation of the Extraction Efficiency of RM-12A-9 to Remove Acephate and Methamidophos Residues From Beans: Lab Project Number: VP-11276: V-96-11276. Unpublished study prepared by Valent Technical Center. 47 p.

43971607 Lai, J. (1996) Validation of the Extraction Efficiency of RM-12A-9 to Remove Acephate and Methamidophos Residues From Lettuce: Lab Project Number: VP-11275: VP11275. Unpublished study prepared by Valent Technical Center. 50 p.

(continued; footnotes follow)

43971608 Lai, J. (1996) Validation of the Extraction Efficiency of RM-12A-9 to Remove Acephate and Methamidophos Residues From Milk and Liver: Lab Project Number: VP-11211: VP11211.Unpublished study prepared by Valent Technical Center. 54 p.

43971610 Lai, J. (1995) Magnitude of Residues of Acephate In/On Mint Hay Following Applications of ORTHENE 75 S: Lab Project Number: V10663: RM-12A-6: V-10663-D. Unpublished study prepared by Valent Technical Center. 364 p.

43971611 Lai, J. (1995) Magnitude of Residues of Acephate In/On Peanuts and Peanut Processed Parts Following Applications of ORTHENE 75 S (Acephate): Lab Project Number: V10671: RM-12A-6: V-94-10671. Unpublished study prepared by Valent Technical Center. 242 p.

44037801 Alam, F.; Burnett, T.; Jalal, M. (1996) Nature of the Residues: Metabolism of (i) (Carbonyl-(carbon-14)) Acephate and (ii) (S-Methyl-(carbon-14)) Acephate in Cotton Plants: Lab Project Number: 94370: VP-10062. Unpublished study prepared by ABC Labs--California. 218 p.

44037802 Lai, J. (1996) Validation of the Extraction Efficiency of RM-12A-9 to Remove Acephate and Methamidophos Residues from Cotton: Lab Project Number: VP-11305: 9600327. Unpublished study prepared by Valent U.S.A Corp. 48 p.

44037803 Lee, D.; McCall, B.; O'Meara, H. (1996) Distribution and Metabolism of (carbon-14)Acephate in Laying Hens: Amended Final Report: Lab Project Number: 94-0098: 6096-94-0098-EF-001: 6-94-0098-EF-001-001. Unpublished study prepared by Ricerca,Inc. 254 p.

44037804 Lai, J. (1996) Validation of the Extraction Efficiency of RM-12A-9 to Remove Acephate and Methamidophos Residues from Eggs and Muscle: Amended Report #1: Lab Project Number:VP-11274: 9600326: V-95-11274. Unpublished study prepared by Valent U.S.A. Corp. 62 p.

[The following MRIDs were not reviewed because amended reports were submitted.]

43971605 Lee, D.; McCall, B.; O'Meara, H. (1996) Distribution and Metabolism of (carbon 14)Acephate in Laying Hens: Lab Project Number: 94-0098: 6096-94-0098-EF-001: 6096-94-0098-EF-000. Unpublished study prepared by Department of Environmental and Metabolic Fate, Ricerca, Inc. 248 p.

(continued; footnotes follow)

43971609 Lai, J. (1996) Validation of the Extraction Efficiency of RM-12A-9 to Remove Acephate and Methamidophos Residues From Eggs and Muscle: Lab Project Number: VP-11274: VP11274. Unpublished study prepared by Valent Technical Center. 60 p.

(*continued; footnotes follow*)