MEMORANDUM

Date: 3/30/00

Subject: PP#9F5046. Thiamethoxam on Canola. Section 3 Registration and Permanent Tolerance Request for Use of Thiamethoxam (Helix Formulation) for Use as a Seed Treatment on Canola Seed Prior to Planting. First Food Use. Residue Chemistry Review: Evaluation of Analytical Method and Residue Data.

DP Barcode: D252021
PC Code: 060109
Prat Case: 290693
Submission Code: S554145
MRID#: 447035-10 thru -12, -15, -16, -20 thru -31, -34 thru -36
447151-06, -13, -14, -16, -17
Trade Name: HELIX Insecticide with Fungicide (contains Thiamethoxam along with 3 fungicides: Difenconazole, Mefenoxam, and Fludioxanil)
40 CFR 180: None (not currently registered)
Class: Insecticide (Neonicotinoid)

From: G. Jeffrey Herndon, Chemist
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Through: Maxie Jo Nelson, Chemist, Secondary Reviewer
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Through: Richard Loranger, Branch Senior Scientist
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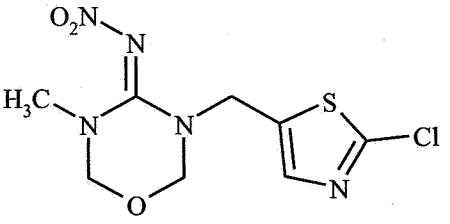
To: Tina Levine/Helene Daniel, P.M. Team #4
Insecticide/Rodenticide Branch
Registration Division (7505C)

Attached is the residue chemistry data review of PP#9F5051 from Novartis (petitioner) requesting the establishment of permanent tolerances for the new neonicotinoid insecticide thiamethoxam and its metabolite CGA-322704 on canola at 0.02 ppm (Limit of Quantitation) and a Section 3 registration to apply thiamethoxam as the Helix formulation to canola seed prior to planting.

This data review was conducted by Dynamac Corporation (contractor) under the supervision of RAB2, HED, and has undergone secondary review and revision within RAB2 to ensure it reflects current HED and OPP policy.

This data review only addresses residue chemistry issues. Product chemistry issues have been the subject of a previous review (memo of A. Smith of TRB/RD, dated 3/18/99, D252040). A human health risk assessment will be the subject of a separate forthcoming HED memo.

THIAMETHOXAM



PERMANENT TOLERANCE PETITION (PP#9F5051) FOR USE ON CANOLA

(DP BARCODE D252021)

INTRODUCTION

Novartis Crop Protection, Inc., has submitted a petition for the establishment of a permanent tolerance for residues of a new insecticide to be used as a combination seed treatment with three fungicides for control of certain insects and diseases of canola. The proposed common name of the insecticide is thiamethoxam (CGA-293343), and the chemical name is 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine. The petitioner is proposing the establishment of a tolerance for residues of thiamethoxam and its major metabolite, N-(2-chloro-thiazol-5-ylmethyl)-N′-methyl-N′′-nitro-guanidine (CGA-322704), converted to parent equivalents in/on:

Canola ..... 0.02 ppm

Thiamethoxam is a new broad spectrum, systemic insecticide with activity against sucking and chewing insects on a wide variety of crops. Thiamethoxam belongs to a new pesticide chemical class known as the neonicotinoids. There are currently no established tolerances for residues of thiamethoxam in/on any plant or animal commodities. However, another petition (PP#9F5046) for the use of thiamethoxam on a wide variety of crops is currently under review by the Agency.

In conjunction with this petition, Novartis is requesting Section 3 registration for a multiple active ingredient (MAI) end-use product composed of thiamethoxam and three fungicides, fludioxonil (PC Code 071503), difenoconazole (PC Code 128847), and mefenoxam (PC Code 113502). The end-use product is a liquid ready to use formulation (RTU; Product Name = Helix™; EPA Reg. No. 100-xxx) containing thiamethoxam at 20.70% or 2.23 lb/gal, difenoconazole at 1.25% or 0.14 lb/gal, mefenoxam at 0.38% or 0.04 lb/gal, and fludioxonil at 0.13% or 0.01 lb/gal. Although the petitioner is proposing use of an MAI, this tolerance petition addresses only the adequacy of the available residue data with respect to thiamethoxam.

No tolerance has been established for residues of difenoconazole in/on canola; however, a petition for a tolerance is pending (PP#9F5045). A tolerance has already been established for fludioxonil residues in/on rape seed (canola) at 0.01 ppm [40 CFR §180.516], and the proposed use rate of fludioxonil in the current petition is ~0.4x the use rate supported by the current tolerance. With regards to mefenoxam, which is the R-enantiomer of metalaxyl, HED has previously concluded (DP Barcode D223261, L. Kutney, 4/24/96) that the existing tolerances for metalaxyl will adequately support uses of mefenoxam provided mefenoxam is used at half the rate currently used for metalaxyl and it is applied in the same manner as metalaxyl. HED has recommended (DP Barcode D214804, W. Cutchin, 12/15/95) establishing a 0.2 ppm tolerance for metalaxyl residues in/on canola resulting from a seed treatment use at rates up to 0.5 oz ai/100 lb seed; this rate is ~4x the use rate proposed for mefenoxam in this petition.

There are 23 volumes of residue chemistry submissions associated with this petition which are evaluated in this document.

CONCLUSIONS

OPPTS 830 Series GLNs: Product Properties

1. Review of technical and end-use product chemistry is under the purview of RD.

OPPTS GLN 860.1200: Proposed Uses

2. The rotational crop restrictions on the proposed label need to be amended. Currently, the proposed label allows wheat to be rotated immediately after planting canola seed treated with thiamethoxam. Typically, the situation would not be encountered (except in cases of crop failure), but the label should be amended to specify a 30-day plantback interval for all crops (except canola, which may be replanted anytime) - see Conclusion 18i. for additional details.

OPPTS GLN 860.1300: Nature of the Residue in Plants

3a. **Pears.** The pear metabolism study is adequate. Following the last of two foliar applications of either [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at 0.27 lb ai/A/application (1.6x the maximum proposed seasonal rate; PP#9F5046), total radioactive residues (TRR) in/on fruit were 0.488 and 0.701 ppm, respectively. Solvent extraction released 83-103% of the TRR in fruit, and nonextractable residues accounted for only ~7-9% of the TRR.

3b. The parent thiamethoxam and its metabolite CGA-322704 were the major components of the residue, accounting for 28-33% and 15-24% of the TRR, respectively. Each of the following minor metabolites were formed from both labeled compounds: CGA-353968

(5-8%TRR), NOA-407475 (2-5%TRR), CGA-265307 (2-5%TRR), desmethyl-CGA-353968 (2-3%TRR), and CGA-322704-hydroxylamine glucoside and CGA-355190 (each 1-3%TRR). In the thiazole-labeled pears, CGA-349208 also accounted for 2% TRR. In addition, NOA-405217, CGA-382191, and NOA-421278 each accounted for 1-2% TRR in pears treated with [oxadiazin-^{14}C]thiamethoxam at a 16x rate (based on the proposed rate for pome fruit in PP#9F5046).

4a. **Cucumbers.** Total radioactive residues in fruit were 0.30 ppm in cucumbers treated with thiazole-labeled [^{14}C]thiamethoxam and 0.32 ppm in cucumbers treated with oxadiazine-labeled [^{14}C]thiamethoxam at 10x the maximum seasonal rate (based on the use proposed in PP#9F5046) and harvested 14 days following the last of repeated applications. Solvent extraction released >80% of the TRR in fruit, and nonextractable residues accounted for only ~6-13% of the TRR (0.02-0.04 ppm).

4b. For the 10x study, thiamethoxam was the principal component of the residue, accounting for 13-14% of the TRR; and the metabolite CGA-353968 accounted 4-10% of the TRR. Minor amounts (0.4-3.6%TRR) of CGA-322704, CGA-355190, and NOA-407475 (thiazole label only) were also formed. The petitioner also isolated from organic and aqueous leaf extracts the following metabolites in minor amounts (<4%TRR): CGA-340575, CGA-349208, desmethyl-CGA-353968, and sugar conjugates of CGA-349208, CGA-353968, and desmethyl-CGA-353968, and NOA-405217.

4c. In the a meeting held on 7/28/99 (see memo of G.J. Herndon dated 8/31/99), the Metabolism Assessment Review Committee (MARC) concluded that the cucumber metabolism study was inadequate due to the low radioactivity present and identified (also see Conclusion 6c.).

5a. **Corn**. The available corn metabolism data is adequate. The metabolic profile of ^{14}C-residues in corn RACs was qualitatively and quantitatively similar following application of either [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam, with the exception of several minor [oxadiazin-^{14}C]-specific metabolites. ^{14}C-Residues were also qualitatively similar between a seed treatment and a soil drench application.

5b. Following treatment of corn seeds with [^{14}C]thiamethoxam (70% WP) at ~1.2 mg ai/seed (0.13 lb ai/A; ~0.65 lb ai/100 lbs of seed), TRRs were 0.104-0.113 ppm in forage harvested 124 days after planting (DAP) and 0.238-0.346 ppm in fodder and 0.015-0.023 ppm in grain harvested at maturity (166 DAP). The principal ^{14}C-residues identified in forage and fodder were comprised of thiamethoxam (3-8% TRR), CGA-322704 (4-12% TRR), NOA-421275 (10-12% TRR), NOA-407475 (7-9% TRR), and CGA-382191 (7-10% TRR), each at 0.007-0.036 ppm. Minor amounts (≤4% TRR) were also detected of CGA-265307, CGA-355190, CGA-353042, and NOA-405217. In grain the only residues detected were thiamethoxam (7-15% TRR) and CGA-322704 (8-10% TRR), each at ≤0.002 ppm.

5c. Following a soil drench application of [^{14}C]thiamethoxam (25% DF) to corn seedlings at 0.43 lb ai/A, TRRs were 0.349-0.402 ppm in forage harvested 89 days posttreatment and 0.88-1.03 ppm in fodder and 0.041-0.080 ppm in grain harvested at maturity (152 days posttreatment). The principal ^{14}C-residues identified in forage were thiamethoxam (28% TRR, 0.098-0.111 ppm), CGA-322704 (16-17% TRR, 0.054-0.069 ppm), NOA-421275 (8-10% TRR, 0.029-0.040 ppm) NOA-407475 (9-10% TRR, 0.035-0.036 ppm), and CGA-382191 (5% TRR, 0.016 ppm), along with minor amounts (≤2% TRR) of CGA-265307, CGA-355190, CGA-353042, and NOA-405217. The same metabolites were identified in fodder, with the relative levels of thiamethoxam (3-5% TRR, 0.032-0.047 ppm) and CGA-322704 (4% TRR, 0.034-0.038 ppm) being lower than in forage. The metabolites NOA-421275 (8-10% TRR, 0.083-0.090 ppm), NOA-407475 (7-8% TRR, 0.068-0.071 ppm), and CGA-382191 (10% TRR, 0.100 ppm) also accounted for a substantial portion of the TRR in fodder, while the remaining metabolites were present at ≤4% of the TRR. In grain, the principal ^{14}C-residues were again thiamethoxam (8-15% TRR) and CGA-322704 (9-16% TRR), each present at ≤0.007 ppm. In addition, low levels (≤4% TRR, ≤0.002 ppm) were also detected in grain of the following metabolites: CGA-265307, CGA-353968, CGA-355190, NOA-421275, NOA-407475, CGA-353042, NOA-405217, and CGA-382191.

5d. Analysis of residual solids following solvent extraction indicated that radioactivity in corn fodder was also associated with pectins (≤0.5% TRR), lignins (1-7% TRR), and cellulose (≤1.4% TRR), and that radioactivity in grain was associated with proteins (3-4% TRR) and starch (7-10% TRR), suggesting that there is some incorporation of residues into natural plant constituents.

6a. The metabolism of thiamethoxam in pears, cucumbers, and corn is similar, although the relative levels of individual metabolites differed among the three crops. To varying degrees, the metabolism of thiamethoxam in each of these crops involves: i) opening of the oxadiazine ring by hydrolysis, ii) loss of the nitro group, iii) hydrolysis of the guanidine moiety to urea derivatives, iv) cleavage of the N-C bridge between the two ring systems, and v) N-demethylation of the oxadiazine ring or its derivatives. Although the exact sequence of these reactions in individual crops is uncertain, metabolites resulting from each of these reactions were present in pears, cucumbers, and corn.

6b. In a meeting held on 7/28/99 (see memo of G.J. Herndon dated 8/31/99), the Metabolism Assessment Review Committee (MARC) concluded that the residue of concern for the proposed seed treatment use on canola is understood. The residue to be regulated (for risk assessment and tolerance setting purposes) is the parent thiamethoxam and its CGA-322704 metabolite.

6c. In the MARC meeting held on 7/28/99, based on the low radioactivity present and identified in the cucumber metabolism study, the Committee recommended that another plant metabolism study be submitted to fulfill the requirement of 3 plant metabolism

studies for OPPTS 860.1300. Based on the uses requested under PP#9F5046 and 9F5051, the Committee recommended that a new plant metabolism study be conducted on a leafy vegetable (one of the representative commodities in either Crop Group 4 or 5).

6d. RAB2 concludes that the two acceptable plant metabolism studies (pears and corn) would cover all proposed seed treatment (wheat, sorghum, barley, canola, cotton, tobacco, leafy (except Brassica) vegetables, Brassica leafy vegetables, cucurbit vegetables, and fruiting vegetables) uses (due to expected non-detectable residues), the proposed foliar treatment uses on cotton and canola (due to the edible portion (oil) undergoing many processing steps which would be expected to degrade or volatilize thiamethoxam residues), foliar uses on tobacco, foliar uses on fruiting (except cucurbit) vegetables (Crop Group 8), foliar uses on cucurbit vegetables (Crop Group 9), foliar and soil treatment of tuberous and corm vegetables (Crop Subgroup 1-C) (due to non-detectable residues in the field trials), and foliar uses on pome fruit (due to acceptable pear metabolism study).

The additional leafy vegetable plant metabolism will need to be conducted by the petitioner, reviewed and found acceptable by the Agency prior to the granting of the following proposed uses of thiamethoxam: foliar treatment of leafy (except Brassica) vegetables (Crop Group 4) and foliar treatment of Brassica leafy vegetables (Crop Group 5).

OPPTS GLN 860.1300: Nature of the Residue in Animals

7a. **Ruminants.** The studies of [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]thiamethoxam metabolism in goats are adequate. More than 74% of the radioactivity in milk and tissues was identified, and the metabolic profile of ^{14}C-residues in milk and tissues was qualitatively and quantitatively similar for the two test substances. Total radioactive residues in milk plateaued within 31-55 hours of initial dosing and reached maximum levels of 1.9-2.3 ppm. Following 3 days of dosing at ~100 ppm, ^{14}C-residue levels were highest in liver (~11 ppm) and kidney (6.6-7.5 ppm). Fat contained the lowest concentration of radioactivity (0.26-0.65 ppm) and muscle contained 2.0-2.3 ppm.

7b. Thiamethoxam and CGA-322704 were the major residues in milk, accounting for 31-37% TRR (0.362-0.545 ppm) and 44-45% TRR (0.514-0.661 ppm), respectively. Metabolite CGA-265307 also accounted for a substantial portion of the residues in milk at 10-18% TRR (0.148-0.208 ppm). Minor metabolites in milk included: desmethyl-CGA-353968 (1.7-2.8% TRR) and NOA-405217 (2.8% TRR). Thiamethoxam was also the major residue in muscle and fat, accounting for 51-54% (1.1 and 1.2 ppm) and 36-52% TRR (0.138-0.278 ppm), respectively, while CGA-322704 accounted for 5-9% TRR in muscle (0.102-0.196 ppm) and 8-12% TRR in fat (0.041-0.048 ppm). Other major residues in fat included NOA-421276 (13-23% TRR) and NOA-421275 (11-13% TRR), and other major residues in muscle included NOA-421276 (5-15% TRR) and MU-12 (7-11% TRR). In kidney, the major residues were thiamethoxam (21-22%TRR; 1.40-1.68 ppm) and NOA-

421275 (16-20 % TRR; 1.24-2.47 ppm). NOA-421276 (13% TRR) and N-5 (12% TRR) were also identified in kidneys along with 10 additional minor metabolites. The metabolism of [^{14}C]thiamethoxam in liver was extensive, with parent being detected at ≤1% TRR. The major residues identified in liver included: NOA-421276 (8-22% TRR), NOA-407475 (9-11% TRR), NOA-421275 (11-13% TRR), and metabolite L-14 (13-25% TRR). Metabolite CGA-322704 was negligible in the initial liver extracts, but was released by microwave-assisted extraction at 6-7% TRR (0.701 and 0.799 ppm).

8a. **Poultry.** The studies of [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]thiamethoxam metabolism in hens are adequate. More than 67% of the radioactivity in eggs and tissues was identified, and the metabolic profile of ^{14}C-residues in eggs and tissues was qualitatively and quantitatively similar for the two test substances. Total radioactive residues in egg whites reached maximum levels of 0.357-0.412 ppm within 48-72 hours, but the highest residues in egg yolks (0.57 ppm) were observed at the last collection interval (72-78 hours). Following 3 days of dosing at ~100 ppm, ^{14}C-residue levels were highest in liver (8-9 ppm) and kidney (4.4-5.2 ppm) and lowest in fat (0.177-0.233 ppm). ^{14}C-Residues in muscle were 0.667-0.929 ppm.

8b. The principal ^{14}C-residues in eggs were CGA-265307, accounting for 45-47% TRR (0.119 and 0.138 ppm) in whites and 54-59% TRR (0.159 and 0.171 ppm) in yolks, and CGA-322704 accounting for 20-25% TRR (0.058 and 0.067 ppm) in whites and yolks. Thiamethoxam was also detected in whites at 2-5% TRR (≤0.013 ppm) and in yolks at 11% TRR (≈0.032 ppm). In egg whites, NOA-4046517 (9-15% TRR) also accounted for a substantial portion of the residue. The major residues in liver were CGA-322704 (34-39% TRR), CGA-265307 (16-20% TRR), and MU3 (12-22% TRR). In the thiazol-label study, NOA-421275 was also detected in liver at 13% TRR. Only trace amounts (0.2% TRR) of parent were detected in liver. Most of the CGA-322704 in liver, >30% TRR (~2.5-3 ppm), was in the fractions solubilized by microwave-assisted extraction. Microwave extraction also released additional amounts of CGA-265307 and NOA-421275. In muscle, the major residues included MU3 (28-39% TRR; 0.261-0.262 ppm) and thiamethoxam (21% TRR; 0.143-0.192 ppm). In the [thiazol-^{14}C] study, NOA-421275 also comprised 11% TRR in muscle. In skin/fat, the major residue was CGA-265307, accounting for 54-58% TRR (0.157-0.210 ppm), with thiamethoxam and CGA-322704 accounting for 5-15% TRR (0.018-0.043 ppm) and 8-9% TRR (0.027-0.028 ppm), respectively.

9a. The metabolism of thiamethoxam in ruminants and poultry is similar. The major pathway of metabolism involves hydrolysis of the oxadiazine ring to form CGA-322704 and subsequent demethylation to produce CGA-265307; loss of the nitro group from these two metabolites also yields NOA-421275 and NOA-421276. Several major metabolites (MU3, L14, and MU12) in both ruminants and poultry also result from the reduction of the nitro group in thiamethoxam or CGA-265307 to a hydrazine, and

subsequent conjugation with acetic or 2-oxo-propionic acids. Separation of the thiazole and oxadiazine rings was only a minor pathway in ruminants and poultry.

9b. In ruminants, the MARC concluded (meeting held on 7/28/99) that the residue of concern is parent + CGA-322704 metabolite. The parent thiamethoxam and the CGA-322704 metabolite accounted for a high percentage of the radioactivity identified in milk and muscle (the commodities with the highest dietary impact for humans) from the lactating goat study. The Committee was potentially concerned about the relatively high levels of metabolites MU-12 and N-5 in kidney and liver. However, after consulting with Alberto Protzel, the Committee concluded that these metabolites, which contain the chloro-thiazole ring but not the nitro group, would not need to be included in the tolerance expression or quantitatively used in a human health risk assessment.

9c. In poultry, based on cursory review of the laying hen metabolism study and dietary burdens, a hen feeding study and establishment of poultry commodity tolerances will not be needed for the proposed uses. If additional uses are requested later which increase the dietary burden enough to require that a laying hen feeding study be conducted, the Committee concluded that the petitioner will need to analyze for the additional metabolite CGA-265307 (in addition to the parent thiamethoxam and CGA-322704 metabolites) based on it being the major residue in eggs and fat and containing the N-nitro group. For the reasons stated under the ruminants section for MU-12 and N-5, the MARC concluded that the metabolite MU-3 would not need to be included in the tolerance expression or quantitatively used in a human health risk assessment.

OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

10a. Novartis HPLC/UV (or MS) Method AG-675 (MRID# 447035-24) is adequate for collecting data on residues of thiamethoxam and CGA-322704 in/on canola and mustard seed. Adequate method validation data were submitted for canola seed (in MRID# 447035-27) and various additional crop matrices. Method AG-675 has been adequately radiovalidated, and has undergone a successful (independent laboratory validation (ILV) trial. The validated limit of quantitation (LOQ) for residues of each analyte is 0.01 ppm in all plant matrices with the exception of fruit juices (0.005 ppm) and grass (0.05 ppm).

10b. Based on achieving adequate recoveries on canola using the LC/MS method (MRID# 447035-27), the petitioner has not adequately explained why the majority of the canola field trial data were analyzed by a LC/MS/MS method. It appears that there may have been some interference problems and, rather than modify or add clean-up steps to eliminate the interference, the petitioner chose to **eliminate 2 clean-up steps** and use a more selective detection system (LC/MS/MS using single ion monitoring). The LC/MS/MS is the proposed enforcement method for canola. RAB2 has reservations about proposing an enforcement method which uses equipment most

enforcement laboratories do not yet have access to in lieu of better clean-up procedures. In addition, no ILV was submitted for the LC/MS/MS method. However, RAB2 will defer to the Analytical Chemistry Branch (ACB) of BEAD for their recommendations concerning this issue. Their comments will be available when they complete the PMV (see Conclusion 10c).

10c. A petition method validation (PMV) request has been submitted to the Analytical Chemistry Branch (ACB) of BEAD (see memo of G.J. Herndon dated 9/28/99). RAB2 requested that ACB use the proposed enforcement methods (MRID# 447035-24 and 447035-27) to validate recovery of thiamethoxam and its metabolite CGA-0322704 from canola, cotton, tomato, spinach, wheat grain, milk, beef liver, and eggs. The results of the PMV request have not been received.

10d. With the submission of a method having both UV and MS detection, the issues of method specificity and confirmatory procedures have been adequately addressed.

OPPTS GLN 860.1340: Residue Analytical Methods - Animal Commodities

11a. Adequate method validation data using animal commodities have been submitted for Novartis HPLC/MS Method AG-675, and the method has undergone a successful ILV trial using milk, eggs, and beef liver. The validated LOQ for residues of thiamethoxam and CGA-322704 is 0.01 ppm each in meat, poultry, and eggs, and 0.005 ppm each in milk. This method has also been adequately radiovalidated using samples of meat and milk from the goat metabolism study. However, additional radiovalidation data are required in order to assess the efficiency of this method in recovering thiamethoxam and CGA-322704 from beef liver. This will be required prior to the establishment of liver and/or meat-byproduct tolerances. In both the ruminant and poultry metabolism studies, microwave extraction was required to release CGA-322704 from liver.

11b. A petition method validation (PMV) request has been submitted to the Analytical Chemistry Branch (ACB) of BEAD (see memo of G.J. Herndon dated 9/28/99). RAB2 requested that ACB use the proposed enforcement methods (MRID# 447035-24 and 447035-27) to validate recovery of thiamethoxam and its metabolite CGA-322704 from canola, cotton, tomato, spinach, wheat grain, milk, beef liver, and eggs. The results of the PMV request have not been received.

11c. Although additional data are required to assess the suitability of Method AG-675 for determining residues in beef liver, this requirement does not impact this petition as animal feeding studies and animal tolerances are not required to support the proposed use on canola.

OPPTS GLN 860.1360: Multiresidue Methods

12a. The petitioner submitted data concerning the recovery of residues of thiamethoxam using FDA multiresidue method protocols (PAM Vol. I). Recovery of thiamethoxam was 50-60% using Protocol D and <30% using Protocol E. Using Protocol C, thiamethoxam obtained adequate detector responses to Section 302 DG5 and DG13 gas-liquid chromatography (GLC) systems. Metabolites CGA-322704 and CGA-265307 were tested using Protocol C, but did not yield adequate detector responses to any of the Section 302 DG5, DG13, and DG18 GLC systems; no further testing was conducted for the metabolites.

12b. In the memos of G.J. Herndon dated 9/28/99, the results of the multiresidue testing were forwarded to FDA (Mark Wirtz) and ACB/BEAD (Francis Griffith).

OPPTS GLN 860.1380: Storage Stability Data

13a. **Plant Commodities**. The submitted two-year storage stability study on thiamethoxam *per se* and one-year interim study on CGA-322704 are adequate pending submission of a detailed description of Method REM 179.03, used to determine residues of each analyte in some study samples. The available data indicate that residues of CGA-322704 and thiamethoxam are stable at ≤- 18 C in apples, corn grain, potato, canola seed, and tomato for up to 1 and 2 years, respectively. HED assumes that Method REM 179.03 is similar to Method REM 179.01, an earlier version of the proposed HPLC/UV enforcement method; however, Method REM 179.03 is capable of determining residues of parent and CGA-322704.

13b. Interim data from an on-going storage stability study are adequate, and indicate that residues of thiamethoxam and CGA-322704 are stable in/on canola oil, corn meal, leaf lettuce, safflower seed, and tomato puree for up to 4 months at -20 C.

13c. Samples of canola and mustard seed from the residue field trials were stored frozen for 2-11 months from collection to analysis. The storage intervals and conditions of the residue studies are adequately supported by the storage intervals depicted in the available storage stability studies.

OPPTS GLN 860.1500: Crop Field Trials

14a. **Canola**. The submitted canola field trial data from six tests conducted in the U.S. are adequate. Combined residues of thiamethoxam and CGA-322704 were below the combined LOQ (<0.02 ppm) in/on 12 samples of canola seed grown from seed treated with thiamethoxam at 500 g ai/100 kg seed (0.5 lb ai/100 lb seed; ~1x the maximum proposed use rate) and harvested at maturity, 87-295 days after planting. These data

support the proposed tolerance of 0.02 ppm for the combined residues of thiamethoxam and CGA-322704 in/on canola seed.

14b. The petitioner submitted additional canola field trials data from 16 tests performed in Canada and 2 tests performed in the U.S. These residue data were not used to determine the adequacy of the proposed tolerance because samples from these tests were analyzed using methods for which the combined LOQ for residues of thiamethoxam and CGA-322704 (0.05 and 0.1 ppm) exceeds the proposed tolerance. In these tests, the combined residues of thiamethoxam and CGA-322704 were below the combined LOQ (<0.05 or <0.1 ppm) in/on 46 samples of canola seed grown from seed treated with thiamethoxam at 400-500 g ai/100 kg seed (0.4-0.5 lb ai/100 lb seed; ~1x) and harvested at maturity, 84-138 days after planting.

15. **Mustard**. Although no tolerance or use has been proposed on mustard grown for seed, the petitioner submitted mustard field trial data which are reviewed with this petition given the similarity of this use with the canola seed treatment. The combined residues of thiamethoxam and CGA-322704 were below the combined LOQ (<0.1 ppm) in/on 10 mustard seed samples grown from seed treated with thiamethoxam at 400 g ai/100 kg seed (0.4 lb ai/100 lb seed) and harvested at maturity, 101-104 days after planting.

OPPTS GLN 860.1520: Processed Food/Feed

16. Residues of thiamethoxam and CGA-322704 were each <0.01 ppm (<LOQ) in/on two canola seed samples grown from seed treated at 1500 g ai/100 kg seed (1.5 lb ai/100 lb seed; ~3x the maximum proposed application rate). As indicated above, the field trials conducted at ~1x also resulted in no quantifiable residues of thiamethoxam. Therefore, no further processing data or tolerances for residues of thiamethoxam and CGA-322704 in processed canola commodities are required. The maximum theoretical concentration factor for canola is 3x.

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

17. For purposes of this petition, ruminant and poultry feeding studies are not required. Based on results from the animal metabolism studies and the maximum theoretical dietary exposure (0.003 ppm) for livestock resulting from the use on canola, there is no reasonable expectation of finite thiamethoxam residues being transferred to animal commodities. Therefore, tolerances for residues in animal commodities are not required at this time.

OPPTS GLN 860.1850: Confined Accumulation in Rotational Crops

18a. Although the confined rotational crop studies were not conducted at 1x the maximum seasonal rate (0.215 lb ai/A/season based on proposed cotton uses in PP#9F5046) for rotated crops, the available studies are acceptable as ^{14}C-residues were sufficiently identified/characterized and the available data allow HED to conclude that limited field rotational crop studies are necessary to support the proposed 120-day plant-back interval (PBI) for rotational crops in PP#9F5046 for foliar uses. Results from the two confined studies were comparable. As expected, ^{14}C-residues resulting from the 200 g ai/ha application were initially higher than residues resulting from the 100 g ai/ha application; however, the relative distribution of ^{14}C-residues among the various RACs was the same in each study. In addition, the metabolic profile for the two studies was similar, although more metabolites were identified in second study due to the higher levels of ^{14}C-residues. These studies indicate that the metabolism of thiamethoxam in rotational crops is similar to the metabolism observed in the primary crops.

18b. Following a soil application of [^{14}C]thiamethoxam at ~100 g ai/ha (0.09 lb ai/A; ~0.4x for cotton foliar use), radioactive residues were generally low (≤0.05 ppm) in plant samples, with the exception of wheat straw. Radioactive residues were lowest in turnip roots (<0.01 ppm) followed by wheat grain (0.006-0.019 ppm) and mustard/spinach leaves (0.012-0.023 ppm). Radioactive residues were 0.008-0.036 ppm in wheat forage and 0.021-0.169 ppm in straw. For each commodity, ^{14}C-residues were generally highest at the 30-day PBI and lowest at the 120-day PBI. ^{14}C-Residues increased slightly in most commodities between the 120- and 365-day PBIs.

18c. The principal metabolites identified in rotational crops were parent, CGA-322704, and CGA-265307. At the 30-day PBI, thiamethoxam was detected in each commodity except wheat straw, with levels being generally highest in turnip tops and mustard (23.3-39.3% TRR, 0.004-0.020 ppm) and lowest in wheat forage and grain (4.8-15.8% TRR, ≤0.004 pm). By the 365-day PBI, residues of thiamethoxam declined to ≤3.3% TRR (≤0.001 ppm) in spinach and 26.3-31.2% TRR (≤0.008 ppm) in turnip tops, and were not detected in any wheat commodities. The metabolite CGA-322704 was also detected in each commodity (except grain) at various PBIs and accounted for 6.4-47.8% TRR (0.001-0.020 ppm). Metabolite CGA-265307 accounted for a substantial portion of the residues in wheat straw (5.1-32.5% TRR, 0.003-0.055 ppm), but was not detected in turnip tops and was a minor component of the residues in mustard, spinach, and wheat forage and grain (2.6-14.6% TRR, ≤0.003 ppm). Other minor metabolites identified in rotational crops included CGA-322704-hydroxylamine-glucoside (1.8-19.6% TRR, ≤0.004 ppm), CGA-353968 (2.6-14.6 % TRR, ≤0.004 ppm), desmethyl-CGA-353968 (3.0-6.9% TRR, ≤0.007 ppm), CGA-359683 (5.6-10.7% TRR; ≤0.004) and CGA-355190 (1.2% TRR, 0.002 ppm).

18d. Following a soil application of [^{14}C]thiamethoxam at ~200 g ai/ha (0.178 lb ai/A; ~0.8x for cotton foliar use), radioactive residues were generally low (≤0.05 ppm) in RACs, with the exceptions of wheat straw from each PBI, and wheat forage and radish tops from the 29-day PBI, and wheat grain from the 104-day PBI. As in the other confined rotational crop study, ^{14}C-residues were lowest in root matrices (≤0.007 ppm) and highest in straw (0.051-0.753 ppm). With the exception of the anomalous results for wheat grain from the 104-day PBI, ^{14}C-residues were greatest at the 29-day PBI and decreased at subsequent PBIs. Total radioactive residues were <0.01 ppm in lettuce, radishes, and wheat grain from the 362-day PBI.

18e. With the exception of several metabolites specific to the [oxadiazin-^{14}C]-label, the metabolic profiles for the two ^{14}C-labels were similar qualitatively and quantitatively. The principal residues identified in lettuce and radish tops from the 29-day PBI included parent (20-25% TRR) and CGA-322704 (7-16% TRR), although substantial portions of the ^{14}C-residue were also accounted for by the following metabolites: CGA-265307 (9% TRR), CGA-353968 (5% TRR), NOA-407475 (5-9% TRR), NOA-421275 (5-8% TRR), NOA-405217 (3-11% TRR), and CGA-382191 (5-6% TRR). The only components present at ≥0.01 ppm were found in radish tops from the 29-day PBI and included: thiamethoxam (≤0.023 ppm), CGA-322704 (0.012 ppm), and CGA-265307 (0.011 ppm). All metabolites were <0.01 ppm in lettuce from the 29-day PBI and in lettuce and radish tops from the 119-day PBI.

18f. The principal ^{14}C-residues identified in wheat forage from the 29-, 104-, and 180-day PBIs, were CGA-322704 (8-31% TRR; ≤0.011 ppm) and NOA-421275 (9-21% TRR, ≤0.023 ppm), and these compounds were present at ≥0.01 ppm only in forage from the 29-day PBI. The other components identified in forage each accounted 1-7% of the TRR (<0.01 ppm) and included: thiamethoxam, CGA-265307, desmethyl-CGA-3523968, NOA-407475, NOA-405217, and CGA-382191. The metabolic profile in wheat straw was qualitatively similar to the profile in forage; however, no individual metabolite comprised a major portion of the TRR and numerous metabolites were present at levels >0.01 ppm. At the 29-day PBI, thiamethoxam accounted for 3-5% TRR and 0.015-0.038 ppm. Metabolites detected at ≥0.01 and accounting for 3-12% TRR included: CGA-322704 (0.042-0.052 ppm), CGA-265307 (0.047-0.064 ppm), NOA-421275 (0.041-0.066), CGA-382191 (0.071 ppm), NOA-407475 (0.023 ppm), desmethyl-CGA-353968 (0.022-0.025 ppm), and NOA-405217 (0.024 ppm). By the 104-day PBI, metabolites present at 0.013-0.027 ppm included: CGA-322704, CGA-265307, NOA-405217, and CGA-382191. No specific metabolites were present at ≥0.01 ppm in straw by the 362-day PBI. The only residues detected in wheat grain from any PBI were thiamethoxam (≤1% TRR), CGA-322704 (≤4% TRR) and CGA-265307 (7% TRR), each of which were present at ≤0.002 ppm.

18g. In a meeting held on 7/28/99 (see memo of G.J. Herndon dated 8/31/99), the Metabolism Assessment Review Committee (MARC) determined that the major residues from the

confined rotational crop studies were the parent thiamethoxam and its CGA-322704 metabolite. CGA-265307 was a major residue still having the N-nitro group in animal feed items (e.g. wheat straw). All three compounds should be analyzed in field rotational crop studies.

18h. The proposed label for Helix specifies the following rotational crop restrictions: treated areas may be replanted immediately following harvest or as soon as practical following the last application with wheat or canola. Barley, cole crops, cotton, cucurbit vegetables, fruiting vegetables, leafy vegetables, pome fruit, sorghum, tobacco, tuberous and corm vegetables may be planted 30 days after the last application of Helix. For all other crops, a 120-day plantback interval must be observed.

18i. The proposed use of Helix as a seed treatment on canola specifies that a maximum rate of 0.4 lb.ai./100 lbs. of seed. Using a typical seeding rate for canola of 10 lbs./A. (source: Bernie Schneider, HED), this equates to 0.04 lbs.ai./A.. The confined rotational crop study for which the petitioner performed identification work was conducted at about 0.09 lb.ai./A. to the soil. Therefore, for the proposed use of thiamethoxam as a seed treatment use of canola, the confined rotational crop study was conducted at about a 2.25X rate. At a 30-day plantback interval, residues of thiamethoxam and CGA-322704 were a maximum of 0.006 ppm in mustard leaves, 0.026 ppm in turnip tops, 0.015 ppm in wheat forage, 0.02 ppm in wheat straw, and 0.003 ppm in wheat grain. The LOQ of the proposed enforcement method is 0.02 ppm (0.01 ppm for each thiamethoxam and CGA-322704). Therefore, based on the exaggerated rate (2.25X) and low residues found, **RAB2 believes that, for the proposed use of thiamethoxam as a seed treatment use on canola, a 30-day plantback interval is appropriate for all crops (except canola, which may be replanted at any time).**

OPPTS GLN 860.1900: Field Accumulation in Rotational Crops

19a. Data from a limited field trial study are under concurrent review by the Agency in conjunction with a separate petition (PP#9F5046) for tolerances of thiamethoxam on various crop commodities.

19b. In a meeting held on 7/28/99 (see memo of G.J. Herndon dated 8/31/99), the Metabolism Assessment Review Committee (MARC) determined that if a field rotational crop study is needed, the parent thiamethoxam plus the CGA-322704 and CGA-265307 metabolites should be analyzed.

Codex Issues

20. As there are no established Codex MRLs established for residues of thiamethoxam in/on canola, a discussion of compatibility with U.S. tolerances is not relevant at this time.

RECOMMENDATIONS

Pending the results of ACB's validation of the canola enforcement method (Conclusions 10b. and 10c.) and the requested changes on the proposed label (Conclusions 2 and 18i.), for the purposes of this Section 3 registration on canola, the residue chemistry requirements have been met. The proposed tolerance of 0.02 ppm (LOQ) for the combined residues of thiamethoxam and its CGA-322704 metabolite on canola seed is appropriate. Any risk issues will be addressed in the human health risk assessment, which will be the subject of a forthcoming memo by HED.

For future uses, additional plant metabolism (Conclusion 4c, 6c, and 6d), poultry metabolism (Conclusion 9c), radiovalidation of animal commodities method (Conclusion 11a), description of Method REM 179.03 and how it differs from REM 179.01 (Conclusion 13a), ACB validation of animal products enforcement methods (Conclusion 11b), and field rotational crop studies analyzing for residues of thiamethoxam, CGA-322704 and CGA-265307 may be needed.

DETAILED CONSIDERATIONS

OPPTS 830 Series GLNs: Product Properties

The review of technical and end-use product chemistry is under the purview of RD.

OPPTS GLN 860.1200: Proposed Uses

The petitioner provided a specimen label for a multiple active ingredient (MAI) end-use product composed of the insecticide thiamethoxam, and three fungicides, fludioxonil, difenoconazole and mefenoxam. The end-use product is a liquid RTU formulation (Product Name = Helix™; EPA Reg. No. 100-xxx) containing 20.70% or 2.23 lb/gal thiamethoxam, 0.13% or 0.01 lb/gal fludioxonil, 1.25% or 0.14 lb/gal difenoconazole, and 0.39% or 0.04 lb/gal mefenoxam. For the purposes of this petition for thiamethoxam, the end-use product will be referred to as a 2.23 lb/gal RTU formulation.

This product is proposed for use as a combination seed treatment on canola. The insecticidal active ingredient, thiamethoxam, provides control of flea beetles and the fungicidal active ingredients provide control of damping off diseases caused by *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., and seed-borne blackleg caused by *Leptosphaeria maculans*.

The RTU formulation is restricted to use in commercial seed treatment plants and is applied as a slurry seed treatment at 0.4 lb ai/100 lb seed for thiamethoxam. The application rates for the fungicides in this RTU formulation are as follows: 0.4 oz ai/100 lb seed for difenoconazole, 0.12 oz ai/100 lb seed for mefenoxam, and 0.03 oz ai/100 lb seed for fludioxonil. The proposed label prohibits the use of treated seed for feed, food, or oil purposes. The following rotational crop restrictions are proposed for the 2.23 lb/gal RTU formulation: (i) treated areas may be replanted immediately to wheat or canola following harvest or as soon as practical following last application; (ii) barley, cole crops, cotton, cucurbit vegetables, fruiting vegetables, leafy vegetables, pome fruit, sorghum, tobacco, and tuberous and corm vegetables may be planted 30 days after the last thiamethoxam application; (iii) for all other crops, a 120-day PBI from the time of canola seeding or last thiamethoxam application must be observed.

Comments: The rotational crop restrictions on the proposed label need to be amended. Currently, the proposed label allows wheat to be rotated immediately after planting canola seed treated with thiamethoxam. Typically, the situation would not be encountered (except in cases of crop failure), but the label should be amended to specify a 30-day plantback interval for all crops (except canola, which may be replanted anytime) - see Confined Rotational Crop section for additional details.

OPPTS GLN 860.1300: Nature of the Residue in Plants

Pears

Novartis submitted data from a study (citation listed below) investigating the metabolism of [^{14}C]thiamethoxam in pears. The in-life phase of this study was conducted at Novartis' Western Research Station (WRS) in Sanger, CA; determinations of TRR in pear samples were conducted at Novartis' Vero Beach Research Center (VBRC), FL, and the analytical analyses were conducted by Novartis' Human Safety Department (NHSD), Greensboro, NC.

> 44703511 Capps, T. (1998) [^{14}C]CGA-293343: Nature of the Residue in Pears: Lab Project Number: ABR-98041: 198-96: ANPHI-96014. Unpublished study prepared by Novartis Crop Protection, Inc. 171 p.

The petitioner conducted studies with [^{14}C]thiamethoxam separately labeled in the thiazole (labeled at the 2- position) and oxadiazine (labeled at the 4- position) rings. Prior to application, the test substances were mixed with blank wettable powder formulation, and diluted with water to form aqueous suspensions; a wetting agent (Silwet L-77®) was also added at a rate of 0.2%. For the 1.6x and 16x treatments, respectively, the [thiazol-2-^{14}C]thiamethoxam had specific activities of 16.5 and 5.3 μCi/mg, and the [oxadiazin-4-^{14}C]thiamethoxam had specific activities of 15.6 and 5.2 μCi/mg; radiochemical purities were >98.5%.

Each test substance was applied to pears twice foliarly at nominal application rates of 150 or 1500 g ai/ha/application for a total of 300 or 3000 g ai/ha (0.27 or 2.68 lb ai/A/season; 1.6x or 16x the maximum proposed rate for pome fruits). Actual total application rates were 304 and 2864 g ai/ha for the [thiazol-^{14}C], and 301 and 2967 g ai/ha for the [oxadiazin-^{14}C]. Each treatment, including one control, consisted of a single pear tree. Applications were made 13 days apart, and samples of mature fruit were collected 15 days after the last treatment (DAT), and leaves were collected at 0, 15, and 28 DAT. After collection, samples were stored at ≤ -16 C and held at WRS for 4-19 days prior to shipment by overnight carrier on dry ice to VBRC.

Total radioactive residues (TRR)

TRR determinations were conducted by VBRC within 1 month of sampling. Samples were ground with dry ice and radioassayed in triplicate by LSC following combustion. The nominal detection limits (LODs) for the radioassays were ~0.001 and ~0.02 ppm for the 1.6x and 16x treatments, respectively. The TRR in/on treated fruit and leaves are presented in Table 1. TRR in [thiazol-^{14}C] and [oxadiazin-^{14}C]leaves and fruit were approximately the same at the 1.6x rate, and ~10x higher at the 16x treatment rate. Samples were stored frozen for up to 15 days at VBRC, and were then shipped to the analytical laboratory (NHSD) by freezer truck or by overnight courier on dry ice.

Table 1. Total radioactive residues found in/on pear fruit and leaves after two applications of [^{14}C]thiamethoxam totaling 0.27 or 2.68 lb ai/A (1.6x and 16x the maximum proposed seasonal rate).

Matrix	Sampling interval (DAT) [a]	Total radioactive residues (ppm) [b]			
		[Thiazol-^{14}C]		[Oxadiazin-^{14}C]	
		1.6x	**16x**	**1.6x**	**16x**
Leaves	0	42.710	573.23	61.205	652.43
	15	40.101	417.78	51.033	450.54
	28	40.487	423.62	37.852	419.75 [c]
Fruit	15	0.488	6.81	0.701	7.07

[a] Sampling intervals are expressed in terms of days after second treatment (DAT).
[b] Data are expressed in [^{14}C]thiamethoxam equivalents and are the average of triplicate analyses.
[c] Average of five analyses.

Storage stability

Extraction and characterization of ^{14}C-residues were conducted at NHSD. Prior to initial extraction for analysis, plant samples were stored frozen (~-20 C) at NHSD for up to 19 days. Sample extracts were stored refrigerated (~4 C) prior to analysis. Based on the date provided for the earliest initial extraction (10/22/96) and the completion date given for the study (6/1/98), the maximum storage interval for extracts was 20 months. The petitioner provided data from 2D-TLC analyses of the initial organosoluble extracts of ^{14}C-thiazole-treated and ^{14}C-oxadiazin-treated fruit samples, and of methanol (MeOH) fractions from re-extractions conducted "towards the end of the identification phase," 8-15 months later. The quantitative and qualitative data suggest that the metabolite profile was stable for the duration of the study. No additional storage stability data are required.

Extraction and hydrolysis of residues

^{14}C-residues were sequentially extracted twice with ACN:water (9:1, v/v) and once with MeOH:water (9:1, v:v), and filtered. The methanolic extract was rotary evaporated to remove the MeOH and combined with the ACN extract. ^{14}C-Residues in the combined extracts were purified using a C_{18} SPE column eluted with ACN:water (8:1, v/v). The eluant was radioassayed, concentrated, and the ^{14}C-residues were separated on a XAD-4 column eluted with water and MeOH. The MeOH fractions were analyzed by RP-HPLC and 2D-TLC.

The aqueous fractions from the 1.6x treatments were low in radioactivity and contained large amounts of sugars and coextractants; therefore, additional aqueous samples were extracted from larger, "bulk," samples for profiling. The aqueous fractions generated (6.8% and 16.7%TRR in [thiazol-^{14}C] and [oxadiazin-^{14}C]1.6x fruit), were separated on an A-25 anion exchange column eluted with a gradient of water:05N KBr into several neutral and acidic peaks each accounting for 1.2-7.7% of the TRR (0.01-0.05 ppm). No further analysis was attempted on the 1.6x

samples. A similar distribution of radioactivity was obtained from A-25 chromatography of the 16x aqueous extracts, and additional efforts at characterization of these ^{14}C-residues are discussed below.

Residual solids from fruit extractions accounted for 4.6-9.3% of the TRR (1.6x, 0.04-0.07 ppm; 16x, 0.33-0.48 ppm). As residues were low in the unextracted solids, representative bulk PES samples from each treatment and label were subjected to sequential enzymatic, acid and/or base hydrolysis using a cellulase/novozym mixture (acetate buffer, pH ~4.5, at 50 C overnight) followed by 1 N and 6 N HCl, and 2 N NaOH hydrolysis (each refluxed overnight). These procedures released an additional ~3-6% of the TRR in fractions containing <3.5% TRR; no further identification of the hydrolysate components was achieved.

The qualitative and quantitative metabolite profiles for each label by treatment (1.6x or 16x) were essentially the same. The distribution of ^{14}C-activity in individual extracts of fruit from the 16x treatment is presented in Table 2.

Table 2. Distribution and characterization/identification of ^{14}C-residues in/on pear fruit harvested 15 days after the last of two foliar treatments with [^{14}C]thiamethoxam totaling 2.68 lb ai/A (16x the maximum proposed seasonal rate).

Fraction	% TRR [a]	ppm	Characterization/Identification
[Thiazol-^{14}C] Pear fruit (TRR = 6.806 ppm) 15-day PHI			
ACN:H_2O/MeOH:H_2O	102.6	6.983	Combined and fractionated into MeOH and aqueous fractions by XAD-4 chromatography
Organic fraction	75.8	5.159	2D-TLC analysis: Thiamethoxam 33.4% TRR 2.274 ppm CGA-322704 20.7% TRR 1.409 ppm CGA-322704-Glu 1.1% TRR 0.075 ppm CGA-353968 8.0% TRR 0.544 ppm Desmethyl CGA-353968 2.9% TRR 0.197 ppm CGA-265307 3.5% TRR 0.238 ppm CGA-355190 2.7% TRR 0.184 ppm
Aqueous fraction	11.3	0.769	Separation on an A-25 anion exchange column resolved: Neutral Peak (Area 2) 0.7% TRR 0.036 ppm Neutral Peak (Area 3) 5.6% TRR 0.289 ppm Areas 5-8 3.8% TRR 0.196 ppm Further attempts at characterization were unsuccessful.
Unextracted	7.0	0.476	A representative bulk PES sample was subjected to sequential enzyme, acid, and base hydrolyses releasing four fractions, each accounting for 0.4-2.9% of the TRR.
[Oxadiazin-^{14}C] Pear fruit (TRR = 7.071 ppm)			
ACN:H_2O/MeOH:H_2O	90.6	6.406	Combined and fractionated into MeOH and aqueous fractions by XAD-4 chromatography
Organic fraction	68.3	4.829	2D-TLC analysis: Thiamethoxam 30.5% TRR 2.157 ppm CGA-322704 14.9% TRR 1.054 ppm CGA-322704-Glu 0.9% TRR 0.064 ppm CGA-353968 8.4% TRR 0.594 ppm Desmethyl CGA-353968 3.0% TRR 0.212 ppm CGA-265307 2.9% TRR 0.205 ppm CGA-355190 2.8% TRR 0.198 ppm
Aqueous fraction	19.5	1.379	A-25 anion exchange column separation resolved: Neutral Peak (Area 2) 5.8% TRR 0.080 ppm Neutral Peak (Area 3) 9.5% TRR 0.131 ppm Acidic Peak (Area 5) 1.7% TRR 0.023 ppm 2D-TLC analysis of an additional bulk aqueous extract purified by LH-20 chromatography resolved: NOA-405217 1.8% TRR 0.127 ppm CGA-382191 1.6% TRR 0.113 ppm NOA-407475 2.0% TRR 0.141 ppm
Unextracted	4.6	0.325	A representative bulk PES sample was subjected to sequential enzyme and acid hydrolyses releasing three fractions, each accounting for 0.4-3.5% of the TRR

[a] Not corrected for recovery

Characterization and Identification of residues

Residues in the purified fruit and leaf extracts were analyzed by TLC and RP HPLC. For the definitive analysis of ^{14}C-residues in the organosoluble extracts of fruit, residues were quantitated by normal phase 2D-TLC on silica gel plates using methyl ethyl ketone/ACN (80:20, v/v) and ethyl acetate (EtOAc)/isopropanol (PrOH)/H_2O/acetic acid (64/24/12/2, v/v). An additional seven solvent systems were used for 1D- and 2D-TLC of select fractions. Reference compounds were visualized under a UV light (254 nm) and ^{14}C-residues were detected using a radioisotopic imaging system. ^{14}C-Residues in the purified extracts were also analyzed and quantified by RP-HPLC using a C_8 column with a linear gradient of ACN:water (95:5, v/v) to MeOH. Unlabeled reference compounds were detected using a UV detector (254 nm) and ^{14}C-residues were detected using an in-line radioactivity monitor and by LSC of collected fractions. ^{14}C-Residues were identified by co-chromatography using RP-HPLC and 2D-TLC. A total of 17 reference standards, including parent, were used for comparison.

Upon further analysis, the petitioner isolated and identified several metabolites in the 16x oxadiazine-labeled aqueous extracts, a fraction that accounted for 19.5% of the TRR (1.379 ppm) in the initial analysis of fruit extracts. Aqueous ^{14}C-residues obtained from an extraction of larger fruit samples were extensively purified, finally by LH-20 chromatography, and subjected to enzyme treatment. Analysis of the resulting aqueous fraction by 2D-TLC resolved three metabolites, NOA-407475, CGA-382191, and NOA-405217, each accounting for ~2% of the TRR in fruit.

In order to confirm identities of certain metabolites, components were isolated and purified from the 16x leaf samples and analyzed by LC/MS and/or ^{1}H-NMR. The identities of the following metabolites were confirmed: thiamethoxam, CGA-355190, CGA-353968, desmethyl-CGA-353968, CGA-322704, CGA-322704 hydroxylamine glucoside, NOA-405217, and CGA-382191. Residue components identified and characterized in fruit are summarized in Table 3.

Conclusions: The pear metabolism study is adequate. Total radioactive residues in fruit were 0.488 ppm in pears treated with thiazole-labeled [^{14}C]thiamethoxam and 0.701 ppm in pears treated with oxadiazine-labeled [^{14}C]thiamethoxam harvested 15 days following the last of two applications at 1.6x the maximum seasonal rate. Solvent extraction released 83-103% of the TRR in fruit, and nonextractable residues accounted for only ~7-9% of the TRR.

The parent thiamethoxam and its metabolite CGA-322704 were the major components of the residue, accounting for 28-33% and 15-24% of the TRR, respectively. Each of the following minor metabolites were formed from both labeled compounds: CGA-353968 (5-8%TRR), NOA-407475 (2-5%TRR), CGA-265307 (2-5%TRR), desmethyl-CGA-353968 (2-3%TRR), and CGA-322704 hydroxylamine glucoside and CGA-355190 (each 1-3%TRR). In the thiazole-labeled pears, CGA-349208 accounted for 2% TRR. In addition, NOA-405217, CGA-382191, and NOA-421278 from 16x treated oxadiazine-labeled pears each accounted for 1-2% TRR.

Table 3. Summary of radioactive residues characterized/identified in pear fruit harvested 15 days after treated with [^{14}C]thiamethoxam at 0.27 or 2.68 lb ai/A (1.6x and 16x the maximum proposed seasonal rate).

	Thiazole Label				Oxadiazine Label			
	1.6x treatment (TRR = 0.488 ppm)		16x treatment (TRR = 6.806 ppm)		1.6x treatment (TRR = 0.701 ppm)		16x treatment (TRR = 7.071 ppm)	
Fraction	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Thiamethoxam	29.3	0.143	33.4	2.274	28.03	0.196	30.5	2.157
CGA-322704	24.3	0.118	20.7	1.409	19.1	0.134	14.9	1.054
CGA-322704-Glu	1.1	0.006	1.1	0.075	1.1	0.008	0.9	0.064
CGA-353968	5.0	0.024	8.0	0.544	6.0	0.042	8.4	0.594
Desmethyl CGA-353968	1.5	0.007	2.9	0.197	1.84	0.013	3.0	0.212
CGA-265307	4.8	0.023	3.5	0.238	1.7	0.013	2.9	0.205
CGA-355190	0.6	0.003	2.7	0.184	1.1	0.008	2.8	0.198
NOA-407475	2.5	0.012	--	--	4.6	0.021	2.0	0.141
CGA-349208	1.9	0.009	--	--	--	--	--	--
NOA-405217	--	--	--	--	--	--	1.8	0.127
CGA-382191	--	--	--	--	--	--	1.6	0.113
NOA-421275	--	--	--	--	1.2	0.006	--	--
Total identified	**71.0**	**0.345**	**72.3**	**4.921**	**64.7**	**0.441**	**68.8**	**4.865**
Unknowns	4.7	0.023	3.4	0.231	0.9	0.006	4.9	0.346
Aqueous [b]	6.8	0.033	11.3	0.769	16.7	0.117	19.5 [d]	1.379
Total identified or characterized	**82.5**	**0.401**	**87.0**	**5.921**	**82.3**	**0.564**	**89.8**	**6.350**
Nonextractable [c]	7.2	0.035	7.0	0.476	9.3	0.065	4.6	0.325

[a] See Attachment 1 for the full chemical name and chemical structure of the identified metabolites.
[b] Separated by A-25 anion exchange chromatography into multiple neutral and acidic components each containing ≤9.5% of the TRR.
[c] Subjected to enzyme, acid and/or base hydrolyses releasing multiple fractions each containing ≤3.5%TRR
[d] Includes components identified as CGA-382191 (1.8%TRR, 0.127 ppm) and NOA-405217 (1.6%TRR, 0.113 ppm).

Cucumbers

Novartis submitted data from a study (citation listed below) investigating the metabolism of [^{14}C]thiamethoxam in cucumbers. The in-life phase of this study was conducted at Novartis' Western Research Station (WRS) in Sanger, CA. Determinations of TRR in cucumber samples were conducted at Novartis' Vero Beach Research Center (VBRC), FL, and the analytical analyses were conducted by Novartis' Human Safety Department (NHSD), Greensboro, NC.

44703512 Carlin, T. (1998) [^{14}C]CGA-293343: Nature of the Residue in Field Grown Cucurbits: Lab Project Number: ABR-98048: 282-95: ANPHI-97003. Unpublished study prepared by Novartis Crop Protection, Inc. 192 p.

The petitioner conducted two studies in 1995-1996 with [^{14}C]thiamethoxam separately labeled in the thiazole (labeled at the 2- position) and oxadiazine (labeled at the 4- position) rings. Prior to application, the test substances were mixed with unlabeled blank wettable powder formulation and diluted with water to form aqueous solutions with specific activities of 40.1-42.3 μCi/mg (0.5x treatments) and 10.8-11.4 (10x treatments), and radiochemical purities of ≥97.7%.

In the 1995 study each test substance was applied to cucumbers twice foliarly at 50 g ai/ha/application, at a retreatment interval of 10 days, for a total of 100 g ai/ha (0.09 lb ai/A/season; 0.5x the maximum proposed rate for cucurbits). Actual total application rates were 98.7 and 91.0 g ai/ha for the [thiazol-^{14}C] and [oxadiazin-^{14}C] treatments, respectively. Samples of mature fruit were collected at 0 and 14 days after the last treatment, and leaves were collected 14 days after the last treatment.

In 1996, an exaggerated rate study was conducted in which each test substance was applied as a soil drench to seedlings at the first true-leaf stage at 1500 g ai/ha (1.34 lb ai/A) followed 42 days later by a broadcast foliar application at 500 g ai/ha for a total of 2000 g ai/ha (1.78 lb ai/A/season; 10x rate). Actual total application rates were 1872 and 1933 g ai/ha for the [thiazol-^{14}C] and [oxadiazin-^{14}C] treatments, respectively. Samples of leaves and fruits were harvested prior to the foliar application at 42 days after the last treatment to provide samples representing the early-season soil application alone; samples of mature fruit, leaves, and roots and stems were also collected 14 days after the foliar application. After collection, samples were stored at ≤ -9 C and held at WRS for 5-25 days prior to shipment by ACDS freezer truck or overnight carrier on dry ice to VBRC.

Total radioactive residues (TRR)

TRR determinations were conducted by VBRC within ~1-2 month of sampling. Samples were ground with dry ice and radioassayed in triplicate by LSC following combustion. The nominal detection limits (LODs) for the radioassays were ~0.003 and ~0.01 ppm for the 0.5x and 10x treatments, respectively. The TRR in/on treated fruit, leaves, and roots and stems are presented in Table 4. TRR in the [thiazol-^{14}C] and [oxadiazin-^{14}C] fruit were approximately the same at the

0.5x rate, and ~10x higher at the 10x treatment rate. Samples were stored frozen for 1-2 months at VBRC, and were then shipped to the analytical laboratory (NHSD) by overnight courier on dry ice.

Table 4. Total radioactive residues found in/on cucumber matrices after treatment with [^{14}C]thiamethoxam.

Matrix	Posttreatment interval (days)	Total radioactive residues (ppm) [a]	
		[Thiazol-^{14}C]	[Oxadiazin-^{14}C]
foliar treatment only (0.09 lb ai/A; **0.5x** rate)			
Leaves	14	1.628	2.198
Fruit	0	0.039	0.039
	14	0.035	0.031
soil drench only (1.34 lb ai/A)			
Leaves	42	16.40	11.03
Fruit	42	0.280	0.383
soil drench + foliar treatment (1.78 lb ai/A; **10x** rate)			
Leaves	14	13.68	11.48
Root and stem	14	3.10	4.41
Fruit	14	0.295	0.323

[a] Data are expressed in [^{14}C]thiamethoxam equivalents and are the average of triplicate analyses.

Storage stability

Extraction and characterization of ^{14}C-residues were conducted at NHSD. Prior to extraction for analysis, plant samples were stored frozen (~-20 C), and sample extracts were stored refrigerated (~4 C) prior to analysis. Sample histories for the analytical phase at NHSD were not provided; however, based on the dates provided for the receipt of samples at NHSD, 9/13/95 and 8/13/96 for the 0.5x and 10x studies, respectively, and experiment termination (11/30/97), the maximum storage interval for samples was approximately 15 or 26 months. The petitioner provided data from RP-HPLC and A-25 analyses of initial organosoluble and aqueous extracts, respectively, of [thiazol-^{14}C] and [oxadiazin-^{14}C] 10x fruit samples conducted in 9/96-10/96, and of same fractions from re-extractions conducted 1 year later. The quantitative and qualitative data suggest that the metabolite profile for each label was stable for up to one year. These data adequately support the 10x study. As relatively similar qualitative and quantitative results were obtained from the 0.5x study, residues of [^{14}C]thiamethoxam may be considered stable in fruit extracts for the duration of the study.

Extraction and characterization of residues

^{14}C-Residues in fruit and leaves were sequentially extracted twice with ACN:water (9:1, v/v) and once with MeOH:water (9:1, v:v), filtered, concentrated to aqueous, and the ^{14}C-residues in the combined extracts were purified using a C_{18} SPE column eluted with ACN:water (8:1, v/v). The eluant was concentrated, and the ^{14}C-residues were separated on a XAD-4 column eluted with water and MeOH. The MeOH fractions were analyzed by RP-HPLC using a C_8 column with a linear gradient of water:ACN (95:5, v/v) to MeOH followed by a rinse of water:ACN (95:5, v/v), and were resolved into six regions of radioactivity (peaks A-F).

The aqueous fractions were separated on an A-25 anion exchange column eluted with a gradient of water to 2 N KBr into three early eluting, neutral, and acidic peaks (Zones A-C), each accounting for <1.0-19.8% of the TRR (<0.001-0.05 ppm) in [thiazol-^{14}C] and [oxadiazin-^{14}C] fruit. For quantitation, eluate fractions were collected and radioassayed by LSC. As similar analyses of the leaf sample extracts produced essentially the same profiles and contained higher residues, they were used for further isolation and identification work, discussed below. Residual solids from fruit extractions accounted for 6.1-33.4% of the TRR (0.002-0.047 ppm), and were not further analyzed.

The distribution of ^{14}C-activity in individual extracts of fruit from the 10x treatment for each label is presented in Table 5. The 10x study includes an early-season soil application and a late-season foliar application, both of which are proposed as methods of application for thiamethoxam. As residues were low in the 0.5x study, and similar qualitative results were obtained from all studies, only the 10x data (14-day PHI) are presented below. The currently proposed label for cucurbits does not specify a PHI for the soil treatment incorporated at planting, and allows a 0-day PHI following two foliar treatments.

Table 5. Distribution and characterization/identification of ^{14}C-residues in/on cucumber fruit harvested 14 days following treatment with an early-season soil drench (1.34 lb ai/A) and a late-season foliar application (0.45 lb ai/A) of [^{14}C]thiamethoxam for a maximum seasonal rate of 1.78 lb ai/A (10x the maximum proposed rate).

Fraction	% TRR	ppm	Characterization/Identification [a]
[Thiazol-^{14}C] Cucumber fruit (TRR = 0.295 ppm) 14-day PHI			
ACN:H_2O/MeOH:H_2O extract	80.66	0.238	Combined and fractionated into MeOH and aqueous fractions by XAD-4 chromatography.
Organic fraction	43.43	0.128	HPLC analysis: Thiamethoxam 13.91% TRR 0.041 ppm CGA-353968 9.69% TRR 0.029 ppm CGA-322704 1.56% TRR 0.004 ppm CGA-355190 0.84% TRR 0.002 ppm NOA-407475 0.13% TRR <0.001 ppm Peak A1 2.83% TRR 0.008 ppm Peak A2 3.24% TRR 0.010 ppm Peak B1 4.28% TRR 0.013 ppm Peak B2 6.39% TRR 0.019 ppm Analysis of comparable leaf extracts indicated that peak Region B contained CGA-349208 and sugar conjugates of CGA-349208, and sugar conjugates of CGA-353968 and desmethyl-CGA-353968, each at 0.3-3.8%TRR (0.08-0.28 ppm).
Aqueous fraction	31.64	0.093	Separation on an A-25 anion exchange column resolved: Early eluting peak (Zone A) 1.75% TRR 0.005 ppm Neutral peaks (Zone B) 16.41% TRR 0.048 ppm Acid peaks (Zone C) 12.77% TRR 0.039 ppm Zone C consisted of seven distinct fractions containing 0.6-3.5%TRR (0.002-0.01 ppm). The radioactivity in these zones were separated into smaller fractions using aqueous extracts from 10x leaves, but was not further identified.
Unextracted	13.3	0.039	Not further analyzed

(Continued; footnotes follow)

Table 5. *(Continued)*.

Fraction	% TRR	ppm	Characterization/Identification [a]
[Oxadiazin-^{14}] Cucumber fruit (TRR = 0.323 ppm) 14-day PHI			
ACN:H_2O/MeOH:H_2O extract	86.9	0.281	Combined and fractionated into MeOH and aqueous fractions by XAD-4 chromatography.
Organic fraction	40.90	0.132	HPLC analysis: Thiamethoxam 12.88% TRR 0.042 ppm CGA-353968 4.18% TRR 0.014 ppm CGA-322704 3.60% TRR 0.013 ppm CGA-355190 0.40% TRR 0.001 ppm Peak A1 7.66% TRR 0.025 ppm Peak A2 6.39% TRR 0.021 ppm Peak B1 2.40% TRR 0.008 ppm Peak B2 2.63% TRR 0.008 ppm Analysis of comparable leaf extracts indicated that Region A contained NOA-407475 and CGA-340575 (each at ≤1.1%TRR, 0.126 ppm), and Region B contained NOA-407475(not quantitated) and sugar conjugates of desmethyl-CGA-353968 (2.5%TRR, 0.28 ppm).
Aqueous fraction	41.30	0.133	Separation on an A-25 anion exchange column resolved: Early eluting peak (Zone A) 11.77% TRR 0.038 ppm Neutral peaks (Zone B) 16.80% TRR 0.054 ppm Acid peaks (Zone C) 11.65% TRR 0.038 ppm Zone C consisted of three distinct fractions containing 2.7-6.2%TRR (0.01-0.02 ppm). Analysis of comparable leaf extracts purified and analyzed by LC/MS indicated that the aqueous fraction contained NOA-407475, CGA-340575, and NOA-405217. Quantitative data were not provided.
Unextractable	6.06	0.020	Not further analyzed

[a] Not corrected for recovery .

Identification of residues

As noted above, HPLC and A-25 profiles of purified extracts of fruit and leaves were similar, and the leaf extracts were used for isolation and identification of the metabolites. Following additional purification steps, thiamethoxam, CGA-353968, CGA-322704, and CGA-355190 were isolated as the major components of the organosoluble extracts, regions C-E, and identified by co-chromatography on normal phase 2D-TLC and/or RP-HPLC with reference standards; their identities were confirmed by LC/MS and ^{1}H-NMR. Thiamethoxam, CGA-322704, and CGA-355190 were also isolated from [thiazol-^{14}C] 10x fruit extracts and identified by co-chromatography with RP-HPLC or 2D-TLC with reference standards. From region F, NOA-407475 was identified by LC/MS as the major metabolite component.

The following systems were used for the co-chromatography identification work. 2D-TLC analyses were conducted using silica gel plates and solvent systems consisting of methylethyl ketone:ACN (80:20, v/v) and EtOAc:PrOH:water (65:25:12, v/v) or $CHCL_3$:toluene:EtOH (33:35:35, v/v) and EtOAc:EtOH:hexane (80:10:10, v/v). RP-HPLC were performed using a C_8 column with a mobile phase gradient of water:ACN (95:5, v/v) to MeOH followed by a rinse of water:ACN (95:5, v/v). For the TLC analyses, reference compounds were detected using UV light, and ^{14}C-residues were detected and quantified using AMBIS or FUJI BAS 1000 radioanalytic imaging systems. For the HPLC analyses, reference compounds were detected using a UV detector (254 nm) and ^{14}C-residues were detected using an in-line radioactivity monitor. A total of 12 reference standards, including parent, were used for comparison.

The following compounds were also identified by LC/MS as components of regions A and B after additional preparative clean-up, cellulase treatment and/or acetylation of these organosoluble peaks: CGA-340575 and NOA-407475 from region A, and sugar conjugates of CGA-349208, CGA-353968, and desmethyl-CGA-353968 from region B. Likewise, LC/MS identified NOA-407475, CGA-340575 and NOA-405217 as components of purified aqueous fractions extracts from leaves. Residue components identified and characterized in fruit are summarized in Table 6.

Conclusions: Total radioactive residues in fruit were 0.30 ppm in cucumbers treated with thiazole-labeled [^{14}C]thiamethoxam and 0.32 ppm in cucumbers treated with oxadiazine-labeled [^{14}C]thiamethoxam at 10x the maximum seasonal rate and harvested 14 days following the last of repeated applications. Solvent extraction released >80% of the TRR in fruit, and nonextractable residues accounted for only ~6-13% of the TRR (0.02-0.04 ppm).

For the 10x study, parent thiamethoxam was the principal component of the residue, accounting for 13-14% of the TRR. The metabolite CGA-353968 accounted 4-10% of the TRR from thiazol- and oxadiazine-labeled cucumbers, respectively. Minor amounts (0.4-3.6%TRR) of CGA-322704, CGA-355190, and NOA-407475 (thiazole label only) were also formed. The petitioner also isolated from organic and aqueous leaf extracts the following metabolites in minor

amounts (<4%TRR): CGA-340575, CGA-349208, desmethyl-CGA-353968, and sugar conjugates of CGA-349208, CGA-353968, and desmethyl-CGA-353968, and NOA-405217.

In the MARC meeting held on 7/28/99, based on the low radioactivity present and identified in the cucumber metabolism study, the Committee recommended that another plant metabolism study be submitted to fulfill the requirement of 3 plant metabolism studies for OPPTS 860.1300.

Table 6. Summary of radioactive residues characterized/identified in cucumber fruit treated with [^{14}C]thiamethoxam as follows: (1) two broadcast foliar treatments at 0.09 lb ai/A/season (0.5x; 14-day PHI); (2) at-planting soil drench only at 1.35 lb ai/A/season (7.5x; 42-day posttreatment interval); or (3) at-planting soil drench + two foliar applications at 1.78 lb ai/A/season (10x; 14-day PHI).

	Thiazole Label						**Oxadiazine Label**					
	0.5x treatment (TRR = 0.035 ppm)		Soil drench alone (TRR = 0.282 ppm)		10x treatment (TRR = 0.295 ppm)		0.5x treatment (TRR = 0.031 ppm)		Soil drench alone (TRR = 0.383 ppm)		10x treatment (TRR = 0.323 ppm)	
Fraction	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]												
Thiamethoxam	16.19	0.006	25.51	0.071	13.91	0.041	6.44	0.002	38.58	0.148	12.88	0.042
CGA-353968	2.25	0.001	1.14	0.003	9.69	0.029	6.64	0.002	5.87	0.022	4.18	0.014
CGA-322704	1.30	0.001	10.24	0.029	1.56	0.004	0.06	<0.001	7.06	0.027	3.60	0.013
CGA-355190	0.70	<0.001			0.84	0.002	--	--	--	--	0.40	0.001
NOA-407475	0.37	<0.001	0.98	0.003	0.13	<0.001	0.13	<0.001	5.10	0.020	--	--
Total identified	**20.81**	**0.007**	**37.87**	**0.106**	**26.13**	**0.077**	**13.27**	**0.006**	**56.61**	**0.217**	**21.06**	**0.07**
Characterized												
Organosoluble Peak A1 [b]	11.04	0.004	12.62	0.035	2.83	0.008	13.4	0.004	9.67	0.037	7.66	0.025
Peak A2	0.39	<0.001	2.60	0.007	3.24	0.010	5.82	0.002			6.39	0.021
Peak B1 [c]	11.05	0.004	5.40	0.015	4.28	0.013	3.15	0.001	11.76	0.045	2.40	0.008
Peak B2			5.80	0.016	6.39	0.019	7.12	0.002	6.48	0.025	2.63	0.008
Aqueous [d] Early Eluting Peak A	0.23	<0.001	0.20	0.001	1.75	0.005	4.98	0.002	4.07	0.016	11.77	0.038
Neutral Peak B	19.83	0.007	4.20	0.012	16.41	0.048	18.43	0.006	9.78	0.037	16.80	0.054
Acid Peak C [e]	8.60	0.003	2.07	0.006	12.77	0.039	10.43	0.003	4.74	0.018	11.65	0.038
Total identified or characterized	**71.95**	**0.025**	**70.76**	**0.198**	**73.80**	**0.218**	**76.6**	**0.026**	**103.1**	**0.395**	**80.36**	**0.262**
Nonextractable	33.4	0.012	13.94	0.039	13.3	0.039	6.54	0.002	12.3	0.047	6.06	0.020

[a] See Attachment 1 for the full chemical name and chemical structure of the identified metabolites.
[b] NOA-407475 was also found in comparable fractions (peak region A) extracted from leaves.
[c] Region B was shown by analysis of comparable leaf extracts to contain the following: NOA-407475, sugar conjugates of CGA-353968 and desmethyl-CGA-353968, and CGA-349208 and sugar conjugates of CGA-349208 (thiazole label only).
[d] NOA-407475, CGA-340575, and NOA-405217 were also found in the aqueous fraction of oxadiazine-labeled leaf extracts.
[e] Composed of 3-7 separate peaks each accounting for <1.0-8.4% of the TRR (<0.001-0.02 ppm).

Corn

Although no uses for thiamethoxam have been proposed for corn, Novartis submitted data from a series of experiments investigating the metabolism of [^{14}C]thiamethoxam in corn following either a seed treatment, soil drench, or stem injection. The in-life and analytical phases of these experiments were conducted by Novartis at their research facilities in St. Aubin and Basel, Switzerland. Results from these studies are reported in:

44703515 Sandmeier, P. (1997) Metabolism of (Thiazol-2-^{14}C) CGA-293343 in Corn: Lab Project Number: CMR 19/97: 764-97: 95PSA41. Unpublished study prepared by Novartis Crop Protection AG. 109 p.

44703516 Sandmeier, P. (1996) Uptake, Distribution and Degradation of CGA-293343 in Field Grown Corn after Seed Treatment with (Thiazol-2-^{14}C) Labeled Material: Lab Project Number: PMR 6/96: 505-96. Unpublished study prepared by Novartis Crop Protection AG. 95 p.

44703520 Sandmeier, P. (1996) Uptake, Distribution and Degradation of CGA-293343 in Field Grown Corn After Seed Treatment with (Oxadiazin-4-^{14}C) Labeled Material: Lab Project Number: PMR 7/96: 506-96. Unpublished study prepared by Novartis Crop Protection AG. 108 p.

44703521 Sandmeier, P. (1997) Metabolism of (Oxadiazin-4-^{14}C) CGA-293343 in Corn: Lab Project Number: CMR 10/97: 763-97: 95PSA40. Unpublished study prepared by Novartis Crop Protection AG. 208 p.

Seed Treatment and Soil Drench. Initially, the petitioner conducted separate studies using [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]labeled thiamethoxam for both a seed treatment and a soil drench application to corn seedlings. Both ^{14}C-labels were diluted with non-labeled thiamethoxam and formulated as a 70% WP for the seed treatment and as a 25% DF for the soil drench treatment. The [^{14}C]thiamethoxam in each formulation had a final specific activity of 54.1 μCi/mg (102,102 dpm/μg) and a radiochemical purity of ≥96%.

For the seed treatments, the 70% WP test substances were moistened with water and slurried with corn seeds. The seeds were allowed to soak in the slurry overnight in the dark. Ten seeds/^{14}C-label were randomly sampled for radioassay, and the remaining seeds were planted in field plots. The application rate was 1.26 and 1.21 mg ai/seed for [thiazol-^{14}C] and [oxadiazin-^{14}C]thiamethoxam, respectively. Based on the plant spacing, the petitioner estimated that these rates were equivalent to 149 and 145 g ai/ha (0.13 lb ai/A). Based on a average of ~150 corn seed/oz and a planting rate of 15 lbs of seed/A, HED estimates that the above rates approximate a seed treatment rate of 0.65 lb ai/100 lbs of seed, or 0.1 lb ai/A.

For the soil drenches, the top 2 cm of soil was removed from around twenty corn seedlings per ^{14}C-label (2 leaf stage; 14 days after planting) growing in field plots. The 25% DF test substances, suspended in water, were applied around each plant, and the soil was replaced. The application rate was 4.13 and 4.05 mg ai/plant for [thiazol-^{14}C] and [oxadiazin-

[^{14}C]thiamethoxam, respectively, equivalent to 488 and 485 g ai/ha (0.43 lb ai/A). These rates were 3.3x the dose rate used for the seed treatments.

Samples of forage were collected from the seed treatments at 14, 33, and 124 days after planting (DAP), and fodder and grain were collected at crop maturity (166 DAP). Forage samples from the soil drench treatment were collected at 89 DAT, and fodder and grain were collected at crop maturity (152 DAP). After sampling, all samples were stored at the analytical laboratory at ≤-18 C.

Stem Injection Experiment. To provide additional material for isolating and identifying ^{14}C-residues in corn matrices, the petitioner also conducted stem injections of corn plants with [^{14}C]thiamethoxam in a greenhouse. In separate tests, 27-day old corn plants were injected with either [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at 1.21 and 1.26 mg ai/plant, respectively. These dose levels corresponded to the rates used for the seed treatment tests. Both ^{14}C-labels had a specific activity of 54.1 µCi/mg (radiochemical purity ≥96%) and were dissolved in dimethylsulfoxide for injection. A total of 16 plants per ^{14}C-label each received 10 µL injections of test material in the stalk at 5 and 10 cm above the soil.

Corn plants were harvested at maturity, 78 days posttreatment, and separated into leaves, stalks, grain, and cobs+husks (not analyzed). Samples were air dried for 16 days prior to being stored at ≤-18 C and radioassayed.

Total radioactive residues (TRR)

Seed Treatment and Soil Drench Experiments. With the exception of the 14-day forage samples, TRRs were determined in triplicate by LSC following combustion. Radioactivity in the 14-day forage sample was calculated as the sum of extracted and non-extracted radioactivity determined by LSC and combustion/LSC, respectively. The LOQ for the radioassays was 0.001 ppm for plant samples. The TRRs in corn RACs are presented in Table 7. ^{14}C-Residues resulting from the application of the two ^{14}C-labels were similar. For both methods of application, TRR levels were lowest in grain and highest in fodder. Levels in forage harvested at 89 or 124 DAT were 2-3x lower than in fodder. In accordance with the respective dose levels, ^{14}C-residues in RACs from the soil drench treatments were 2.5-3.6x higher than in the corresponding RACs from the seed treatments.

Stem Injection Experiments. TRRs were determined in triplicate by LSC following combustion, and the LOQ for the radioassays was 0.001 ppm. As in the seed and soil drench treatments, the distribution of radioactivity in corn plants following stem injection was similar for the two ^{14}C-labels. At maturity (78 days posttreatment), 63-64% of the applied radioactivity had been translocated into the leaves, 2-5% remained in the stalks, and only 0.2-0.3% had been translocated into the grain. The TRRs in stalks, leaves, and grain were 0.868, 66.7, and 0.058 ppm, respectively, for [thiazol-^{14}C]-treated plants, and were 1.697, 59.1, and 0.035 ppm, respectively, for [oxadiazin-^{14}C]-treated plants.

Table 7. Total radioactive residues found in/on corn RACs following a seed treatment or soil drench of [^{14}C]thiamethoxam [a].

Matrix	Sampling interval (days)	Total radioactive residues (ppm) [b]			
		[Thiazol-^{14}C]		[Oxadiazin-^{14}C]	
		seed treatment	soil drench	seed treatment	soil drench
Seeds	0	4174.0	Not applicable (NA)	3761.6	NA
forage	14	73.8	NA	NA	NA
	33	13.777	NA	18.152	NA
	89 [c]	NA	0.402	NA	0.349
	124 [c]	0.113	NA	0.104	NA
fodder	166/152 [d]	0.346	0.882	0.238	1.030
grain	166/152 [d]	0.023	0.080	0.015	0.041

[a] Application rates were 1.2 mg ai/plant (~0.13 lb ai/A) for the seed treatments and 4.1 mg ai/plant (~0.43 lb ai/A) for the soil drenches.
[b] Data are expressed in [^{14}C]thiamethoxam equivalents and are the average of triplicate analyses.
[c] Forage samples collected at 89 and 124 DAT reflect a typical forage harvest for corn.
[d] Fodder and grain samples were collected at maturity, 166 DAT for seed treatments and 152 DAT for soil drenches.

Storage stability data

Seed Treatment and Soil Drench Experiments. Samples were extracted for analysis within 2-45 days of collection. Sample extracts were stored at ≤8 C and were initially profiled by TLC within 2-47 days of extraction. The analytical phase of the studies was completed within one year of the initial analyses. To demonstrate the stability of ^{14}C-residues in sample extracts over the course of the analytical phase, the petitioner presented 2D-TLC chromatograms and quantitative data from the analysis of forage, fodder and grain extracts. These extracts were originally analyzed by 2D-TLC within 2-45 days of extraction and were then reanalyzed 94-236 days later. Both the TLC chromatograms and the quantitative data indicate that ^{14}C-residues in extracts were stable over the course of the study.

Stem Injection Experiments. All samples from the [oxadiazin-^{14}C]-treated corn and the grain sample from the [thiazol-^{14}C]-treated corn were extracted for analysis after 10 days of frozen storage and were profiled by TLC within 7 days of extraction. However, the leaf sample from the [thiazol-^{14}C]-treated corn was stored frozen for 171 days prior to extraction and the extract was held for another 45 days prior to chromatographic analysis. The available storage stability data from the previous experiments support the stability of ^{14}C-residues in the stem injection studies. No additional storage stability data are required to support the corn metabolism studies.

Extraction and hydrolysis of residues

Seed Treatment and Soil Drench Experiments. Extraction and characterization of ^{14}C-residues were conducted at the petitioner's analytical laboratory in Basel, Switzerland. ^{14}C-Residues were extracted repeatedly with MeOH:water (8:2, v:v), filtered, combined and concentrated to remove organic solvents. The methanolic extracts from forage were then directly analyzed by 2D-TLC. Methanolic extracts from fodder and grain were first partitioned between dichloromethane (DCM) and water prior to TLC analysis. In addition, the aqueous fraction of grain from the soil drench treatments were further fractionated using an anion exchange column eluted sequentially with water and MeOH. The resulting fractions were analyzed by TLC. With the exception of the residual solids from forage of soil-drenched plants, ^{14}C-residues remaining in solvent-extracted solids were soxhlet extracted with MeOH.

The extraction and fractionation of ^{14}C-residues in forage, fodder and grain from seed treatment and soil drench treatment are presented in Tables 8 and 9, respectively. The distribution of ^{14}C-residues was similar between the two application methods, but differed between the two ^{14}C-labels for the fodder and grain samples. For forage samples, solvent extraction released 74-85% of the TRR, and the subsequent soxhlet extraction with MeOH released an additional 2-3% of the TRR, leaving 12-25% of the TRR (0.22-0.063 ppm) unextracted. For fodder and grain, ^{14}C-residues were more readily extracted from [oxadiazin-^{14}C]-treated samples than from [thiazol-^{14}C]-treated samples. Regardless of the application method, solvent extraction released 47-52% TRR from fodder and 39-43% TRR from grain of [thiazol-^{14}C]-treated plants and 66-68% TRR from fodder and 71-77% TRR from grain of [oxadiazin-^{14}C]-treated plants. Subsequent soxhlet extraction with MeOH released only $\leq$3.5% of the TRR.

To further characterize ^{14}C-residues remaining in solvent-extracted solids, the petitioner used residual solids of fodder and grain from the soil drench treatments. Residual solids accounted for 33-42% of the TRR (0.347-0.374 ppm) in fodder and 26-62% of the TRR (0.011-0.049 ppm) in grain for both ^{14}C-labels from this treatment.

Residual solids from fodder were further extracted by boiling in water overnight, which released an additional 15-16% TRR (Table 10). The aqueous extract was acidified (pH 2) with HCl, concentrated, and diluted with ethanol (EtOH) to precipitate pectins (0.4-0.5% TRR), and the resulting aqueous fraction (12-14% TRR) was analyzed by 2D-TLC. The remaining solids were then extracted by refluxing in 2N NaOH for 3 hours. Alkaline hydrolysis released 18-25% of the TRR and 0.5-1.4% of the TRR remained in the solids (cellulose). The hydrolysate was acidified with HCl to pH 1 and cooled to precipitate lignins (1-7% TRR). The solubilized ^{14}C-residues were then neutralized (pH 7.7) and fractionated using a ion exchange column eluted sequentially with water, 50% MeOH, and MeOH. The resulting aqueous and methanolic eluants (2.0-6.2% TRR) were analyzed by TLC.

Table 8. Extraction and distribution of ^{14}C-residues in forage, fodder, and grain from corn treated with [^{14}C]thiamethoxam as a seed treatment at ~1.2 mg ai/seed (~0.13 lb ai/A).

Fraction	[Thiazol-^{14}C]Thiamethoxam					
	Forage (0.113 ppm)		Fodder (0.346 ppm)		Grain (0.023 ppm)	
	%TRR [a]	ppm [b]	%TRR	ppm	% TRR	ppm
MeOH/water	73.6 [c]	0.083	47.4 [c]	0.164	38.7	0.009
DCM	-- [d]	--	14.6 [c]	0.051	16.1 [c]	0.003
Aqueous	--	--	35.5 [c]	0.123	19.6	0.005
MeOH soxhlet	1.6	0.002	1.9	0.007	3.1	<0.001
Solids	25.0	0.028	49.1	0.170	65.4	0.015
Fraction	[Oxadiazin-^{14}C]Thiamethoxam					
	Forage (0.104 ppm)		Fodder (0.238 ppm)		Grain (0.015 ppm)	
	%TRR	ppm	%TRR	ppm	% TRR	ppm
MeOH/water	74.2 [c]	0.077	67.8 [c]	0.161	71.0	0.001
DCM	--	--	10.3 [c]	0.025	27.1 [c]	0.004
Aqueous	--	--	58.4 [c]	0.139	40.5 [c]	0.006
MeOH soxhlet	2.8	0.003	3.5	0.008	3.5	<0.001
Solids	21.3	0.022	30.9	0.074	24.8	0.004

[a] %TRR values are not corrected for recoveries.
[b] Expressed as [^{14}C]thiamethoxam equivalents.
[c] Fraction analyzed by 2D-TLC.
[d] -- = not applicable.

Residual solids from corn grain were extracted overnight with 0.05 N NaOH and centrifuged, releasing an additional 7-10% TRR (Table 11). The basic extract was neutralized (pH 6) with HCl and diluted with EtOH to precipitate proteins (3-4% TRR), and the remaining aqueous fraction (4-7% TRR) was partitioned between DCM (0.1% TRR) and water (4-6% TRR). No further analysis was conducted on the DCM fractions or on the aqueous fraction from [oxadiazin-^{14}C] labeled grain. However, the aqueous fraction from [thiazol-^{14}C] labeled grain was further fractionated using a silica gel column, and the initial eluant fraction (4.2% TRR) was analyzed by 2D-TLC.

The remaining corn grain solids were then hydrolyzed by refluxing in 1N HCl for 17 hours. Acid hydrolysis solubilized 18-47% of the TRR, and the remaining solids accounted for 3-4% of the TRR. For the [oxadiazin-^{14}C] labeled grain, the acid hydrolysate was neutralized and derivatized by refluxing with phenylhydrazine-hydrochloride, cooled, and filtered. The resulting glucosazone was filtered, redissolved in MeOH/water and recrystallized. For the [thiazol-^{14}C] labeled grain, the acid hydrolysate was neutralized and partitioned between DCM (2.5% TRR) and water (48.0% TRR). The DCM fraction was analyzed by 2D-TLC and the aqueous fraction was derivatized to yield a glucosazone fraction as above. An additional aliquot of the aqueous ^{14}C-residues were also fractionated using an anion exchange column eluted with water and 0.5N HCl:MeOH (1:1, v/v), resulting in one aqueous fraction (9% TRR) and two HCl/MeOH fractions

(32% and 1% TRR). The principal HCl/MeOH fraction (32% TRR) was analyzed by TLC before and after methylation and acetylation.

Table 9. Extraction and distribution of ^{14}C-residues in forage, fodder and grain from corn seedlings treated with [^{14}C]thiamethoxam as a soil drench at 4.1 mg ai/plant (~0.43 lb ai/A).

Fraction	[Thiazol-^{14}C]Thiamethoxam					
	Forage (0.402 ppm)		Fodder (0.882 ppm)		Grain (0.080 ppm)	
	%TRR [a]	ppm [b]	%TRR	ppm	% TRR	ppm
MeOH/water	84.7 [c]	0.340	51.6 [c]	0.455	43.3	0.035
DCM	-- [d]	--	12.7 [c]	0.112	19.6 [c]	0.016
Aqueous	--	--	40.0 [c, e]	0.353	20.4 [c]	0.016
Anion exchange Aqueous-1	--	--	--	--	6.0 [c, e]	0.005
Aqueous-2	--	--	--	--	5.4 [c]	0.004
MeOH	--	--	--	--	7.1 [c]	0.006
MeOH soxhlet	--	--	2.0	0.018	3.0	0.002
Solids	15.7	0.063	42.4	0.374	61.5	0.049
Fraction	[Oxadiazin-^{14}C]Thiamethoxam					
	Forage (0.349 ppm)		Fodder (1.030 ppm)		Grain (0.041 ppm)	
	%TRR	ppm	%TRR	ppm	% TRR	ppm
MeOH/water	84.6 [c]	0.295	65.9 [c]	0.679	76.7	0.031
DCM	--	--	10.3 [c]	0.106	33.7 [c]	0.014
Aqueous	--	--	55.9 [c, e]	0.578	40.6 [c]	0.017
Anion exchange Aqueous-1	--	--	--	--	5.7 [c]	0.002
Aqueous-2	--	--	--	--	17.9 [c, e]	0.007
MeoH	--	--	--	--	16.1 [c]	0.007
MeOH soxhlet	--	--	2.7	0.028	2.9	0.001
Solids	12.2	0.043	33.7	0.347	26.4	0.011

[a] %TRR values are not corrected for recoveries.
[b] Expressed as [^{14}C]thiamethoxam equivalents.
[c] Fraction(s) analyzed by 2D-TLC.
[d] -- = not applicable.
[e] Enzymatic hydrolysis with β-glucosidase and cellulase had no affect on the TLC profile.

Table 10. Fractionation of ^{14}C-residues in solvent extracted solids of fodder from corn seedlings treated with [^{14}C]thiamethoxam as a soil drench at 4.1 mg ai/plant (~0.43 lb ai/A).

Fraction	[Thiazol-^{14}C] Fodder (0.882 ppm)		[Oxadiazin-^{14}C] Fodder (1.030 ppm)	
	%TRR [a]	ppm [b]	%TRR	ppm
MeOH/water [c]	51.6	0.455	65.9	0.679
MeOH soxhlet	2.0	0.018	2.7	0.028
Solids	42.4	0.374	33.7	0.347
Boiling H_2O	15.9	0.140	14.7	0.151
Acidic aqueous fraction [d]	14.3 [e]	0.126	11.6 [f]	0.119
Acidic precipitate (pectins)	0.4	0.003	0.5	0.006
Solids	NR [g]	--	NR	--
2N NaOH hydrolysate	24.7	0.218	17.7	0.182
Acidic precipitate (lignin)	7.3	0.064	1.2	0.012
Acidic aqueous fraction	11.3	0.100	12.1	0.125
ion exchange fractions aqueous [d]	4.5 [h]	0.040	6.2 [h]	0.064
50% MeOH [d]	2.5 [i]	0.022	2.1 [i]	0.022
MeOH [d]	2.5 [i]	0.022	2.0 [i]	0.022
MeOH	0.4	0.003		
Solids (cellulose)	1.4	0.012	0.5	0.006

[a] %TRR values are not corrected for recoveries.
[b] Expressed as [^{14}C]thiamethoxam equivalents.
[c] Fractionation of solvent extractable ^{14}C-residues is reported in Table 9.
[d] Analyzed by 2D-TLC.
[e] Detected minor amounts of thiamethoxam (0.6% TRR), CGA-355190 (0.4% TRR), and CGA-322704\CGA-353968 (0.9% TRR), along with 6 unknown fractions each at ≤3.8% TRR (≤0.034 ppm).
[f] Detected minor amounts of thiamethoxam (0.5% TRR), CGA-355190 (0.2% TRR), CGA-382191 (2.3% TRR), NOA-407475 (0.5% TRR), and CGA-322704\CGA-353968 (0.7% TRR), along with 7 unknown fractions each at ≤1.8% TRR (≤0.019 ppm).
[g] NR = not reported.
[h] Polar fraction not resolved by TLC analysis.
[i] 2D-TLC analyses isolated 6-9 unknown regions of radioactivity each accounting for ≤0.8%TRR (≤0.008 ppm).

Table 11. Fractionation of ^{14}C-residues from solvent extracted solids of grain from corn seedlings treated with [^{14}C]thiamethoxam as a soil drench at 4.1 mg ai/plant (~0.43 lb ai/A).

Fraction	[Thiazol-^{14}C] Grain (0.080 ppm)		[Oxadiazin-^{14}C] Grain (0.041 ppm)	
	%TRR [a]	ppm [b]	%TRR	ppm
MeOH/water [c]	43.3	0.035	76.7	0.031
MeOH soxhlet	3.0	0.002	2.9	0.001
Solids	61.5	0.049	26.4	0.011
0.05N NaOH	10.3	0.008	6.7	0.003
EtOH precipitate (proteins)	3.6	0.003	2.5	0.001
Acidic aqueous	6.7	0.005	4.0	0.002
DCM	0.1	<0.001	0.1	<0.001
Aqueous	6.2 [d]	0.005	4.0	0.002
Solids	NR [e]	--	NR	--
1N HCl hydrolysate	47.1	0.038	17.7	0.007
DCM	2.5 [f]	0.002	NA [g]	--
Aqueous	48.0 [h]	0.038		
Filtrate	40.0	0.032	11.3	0.005
Glucosazone (starch)	9.6	0.008	6.8	0.003
Solids	4.4	0.004	2.5	0.001

[a] %TRR values are not corrected for recoveries.
[b] Expressed as [^{14}C]thiamethoxam equivalents.
[c] Fractionation of solvent extractable ^{14}C-residues is reported in Table 9.
[d] Fractionated by silica gel column chromatography into 3 fractions accounting for 4.2, 0.7, and 0.4% TRR. 2D-TLC analysis of the initial MeOH/H_2O/HCOOH fraction (4.2% TRR) showed dispersed regions of radioactivity.
[e] NR = not reported.
[f] 2D-TLC analysis detected thiamethoxam accounting for ≈1.7% TRR.
[g] NA = not applicable.
[h] Fractionated using an anion exchange column into an aqueous fraction (8.7% TRR, 0.007 ppm) and two HCl:MeOH fractions (32.3 and 0.7% TRR; 0.026 and <0.001 ppm). The largest fraction (32.3% TRR) was analyzed by TLC before and after methylation and acetylation. No specific metabolites were identified.

Stem Injection Experiments. Extraction and initial characterization of ^{14}C-residues in leaves and grain were conducted in the same manner as for the seed treatment and soil drench studies. ^{14}C-Residues were extracted repeatedly with MeOH:water (8:2, v:v), filtered, combined and concentrated to remove organic solvents. The methanolic extracts were then directly analyzed by 2D-TLC. In addition, extracted ^{14}C-residues were subjected to a number of different purification procedures in order to provide metabolites for identification purposes.

Characterization and identification of residues

Radioactive residues in solvent extracts and in DCM and aqueous fractions were analyzed and quantified by 2D-TLC using silica gel plates with tetrahydrofuran:MeOH:formic acid:H_2O (60:35:1:4) and chloroform:MeOH:formic acid:H_2O (75:20:4:2). ^{14}C-Residues were detected using a radioanalytic imaging system and quantified by LSC. With two exceptions, reference compounds were detected using UV light; CGA-353042 and NOA-405217 were visualized using a reagent specific for visualizing guanidines.

Solvent extracts from leaves of stem-injected plants (both ^{14}C-labels) and extracts of fodder from the [oxadiazin-^{14}C]soil-drenched plants were used for isolating metabolites for identification. Generally, individual metabolites were isolated using a combination of solvent partitioning, column chromatography (silica gel, C_{18}, and anion exchange), preparative TLC, and HPLC. Isolated metabolites were identified by comparison with reference standards using normal phase 2D-TLC and reverse-phase 1D-TLC, and the identities of the major metabolites were confirmed by NMR and/or MS analysis. The following metabolites were isolated from [oxadiazin-^{14}C]fodder from the soil drench treatment and identified by MS: NOA-407475, CGA-382191, NOA-421275, and CGA-353042. The following metabolites were isolated from [oxadiazin-^{14}C]leaves from the stem injection treatment and identified by MS: thiamethoxam, CGA-322704, CGA-265307, CGA-353968 and NOA-405217. Metabolite CGA-355190 was also identified in leaves of stem injected plants by cochromatography with the same fraction isolated from a wheat cell culture, in which the metabolite was identified by MS; and the metabolite desmethyl-CGA-353968 was identified by cochromatography with the same metabolite (metabolite 4U), which had been identified in a rat metabolism study. The minor thiazole-specific metabolites, CGA-359683, CGA-309335, and CGA-349208, were isolated from the leaves of [thiazol-^{14}C] stem injected plants and were identified by cochromatography with reference standards. Isolated metabolites were also cochromatographed with plant extract fractions to verify that isolated metabolites were not an artifact of the extraction and isolation procedures.

The characterization and identification of solvent-extractable ^{14}C-residues in corn RACs following a seed treatment or a soil drench application with [^{14}C]thiamethoxam are summarized in Tables 12 and 13, respectively. The metabolic profile of ^{14}C-residues in corn RACs was qualitatively and quantitatively similar for the two ^{14}C-labels, with the exception of the [oxadiazin-^{14}C]label specific metabolites, CGA-353042, NOA-405217, and CGA-382191. ^{14}C-Residues were also qualitatively similar between the seed treatment and the soil drench applications.

Following application of [^{14}C]thiamethoxam as a seed treatment at ~0.13 lb ai/A, the principal ^{14}C-residues identified in forage harvested 124 days after planting included thiamethoxam (8% TRR), CGA-322704 (10-12% TRR), NOA-421275 (12% TRR), and NOA-407475 (8-9% TRR), each at 0.008-0.014 ppm. Lower levels of CGA-265307 (2-3% TRR, ≤0.003 ppm) and CGA-355190 (≤1.4% TRR, ≤0.002 ppm) were also detected in forage of both ^{14}C-labels, and the oxadiazin-specific metabolites CGA-353042, NOA-405217, and CGA-382191 were present at 3-8% of the TRR in forage from the [oxadiazin-^{14}C]treated plants. A similar metabolite profile was observed in fodder harvested at maturity (166 DAP), although the relative levels of

thiamethoxam (3-4% TRR, ≤0.015 ppm) and CGA-322704 (4% TRR, ≤0.015 ppm) were lower than in forage, while the relative levels of the other metabolites remained fairly constant. Total radioactive residues in grain were low and the only residues identified were thiamethoxam (7-15% TRR) and CGA-322704 (8-10% TRR), each at ≤0.002 ppm.

Following application of [^{14}C]thiamethoxam as a soil drench to seedlings at ~0.43 lb ai/A, the principal ^{14}C-residues identified in forage harvested 89 days posttreatment were the same ones identified in forage from the seed treatment, except that thiamethoxam (28% TRR, 0.098-0.111 ppm) and CGA-322704 (16-17% TRR, 0.054-0.069 ppm) were present at higher relative and absolute levels. The relative levels of NOA-421275 (8-10% TRR) and NOA-407475 (9-10% TRR) were the same although absolute residue levels were higher (0.029-0.040 ppm). The same metabolite profile was again seen in fodder, with the relative levels of thiamethoxam (3-5% TRR) and CGA-322704 (4% TRR) being lower than in forage and the relative levels NOA-421275 (8-9% TRR) and NOA-407475 (7-8% TRR) remaining constant. In the soil drench treatment, metabolite CGA-382191 (5-10% TRR) also accounted for a substantial portion of the TRR in forage and fodder, and the remaining metabolites were present at ≤4% of the TRR. In grain, the principal ^{14}C-residues were again thiamethoxam (8-15% TRR) and CGA-322704 (9-16% TRR), each present at ≤0.007 ppm. Low levels (≤4% TRR) were also detected in grain of the following metabolites: CGA-265307, CGA-353968, CGA-355190, NOA-421275, NOA-407475, CGA-353042, NOA-405217, and CGA-382191.

In the stem injection experiments, the metabolite profile was again similar between the two ^{14}C-labels, with the exception of a few minor [thiazol-^{14}C] or [oxadiazin-^{14}C] specific metabolites (Table 14). The metabolites were the same as those previously detected in samples from the seed and soil drench treatments, although the relative portion of each metabolite was different. In leaves, thiamethoxam (48-52% TRR) was the principal residue detected at maturity, followed by CGA-322704 (12% TRR) and NOA-407475 (4-6% TRR); all other metabolites were present at ≤3.6% of the TRR.

In both the seed treatment and soil drench experiments, a substantial amount of the radioactivity remained in the solid fractions of fodder and grain following solvent extraction. Residual radioactivity in solids accounted for 31-49% of the TRR (0.074-0.374 ppm) in fodder and 25-65% of the TRR (0.004-0.049 ppm) in grain. For fodder, residual radioactivity was adequately released by boiling water (15-16%) and base hydrolysis (2N NaOH; 18-25% TRR). Analysis of the hot water extracts detected minor amounts (<1% TRR) of thiamethoxam and its principal metabolites, along with CGA-382191 at 2.3% TRR and minor unknown components each at ≤3.8% TRR (Table 10). No metabolites were identified in the base hydrolysate, although fractionation and analysis of the hydrolysate indicated that the base-solubilized residues were comprised of numerous polar unknowns. In addition, minor amounts of the radioactivity in fodder solids were characterized as being associated with pectins (≤0.5% TRR), lignins (1.2-7.3% TRR), and cellulose (≤1.4% TRR), suggesting some incorporation of radioactivity into natural plant compounds.

In grain, residual radioactivity in solids was adequately released by mild base extraction (7-10%) and acid hydrolysis (1N HCl; 18-47% TRR). Analysis of the base extract detected polar diffused regions of radioactivity; no metabolites were identified (Table 11). Analysis of the acid

hydrolysate detected minor amounts of thiamethoxam (≤1.7% TRR), but no other specific metabolites were identified in the hydrolysate. The majority of ^{14}C-residues released by acid hydrolysis were comprised of polar unknowns that cochromatographed with the plant material. In addition, 3-4% of the TRR in grain solids was shown to be associated with proteins and 7-10% of the TRR was identified as being incorporated into glucose.

Conclusions: The available corn metabolism data is adequate. The metabolic profile of ^{14}C-residues in corn RACs was qualitatively and quantitatively similar for the two ^{14}C-labels, with the exception of the [oxadiazin-^{14}C]label specific metabolites, CGA-353042, NOA-405217, and CGA-382191. ^{14}C-Residues were also qualitatively similar between the seed treatment and the soil drench applications.

Following seed treatment, the principal ^{14}C-residues identified in forage and fodder were comprised of thiamethoxam (3-8% TRR), CGA-322704 (4-12% TRR), NOA-421275 (10-12% TRR), NOA-407475 (7-9% TRR), and CGA-382191 (7-10% TRR). Minor amounts (≤4% TRR) were also detected of CGA-265307, CGA-355190, CGA-353042, and NOA-405217. In grain the only residues identified were thiamethoxam (7-15% TRR) and CGA-322704 (8-10% TRR), each at ≤0.002 ppm.

The same metabolites as above were identified in forage and fodder following a soil drench application to corn seedlings. The principal ^{14}C-residues were again thiamethoxam (3-28% TRR), CGA-322704 (4-17% TRR), NOA-421275 (8-10% TRR), NOA-407475 (7-10% TRR), and CGA-382191 (5-10% TRR), with the remaining metabolites each accounting for ≤4% of the TRR. In grain, the principal ^{14}C-residues were again thiamethoxam (8-15% TRR) and CGA-322704 (9-16% TRR), each present at ≤0.007 ppm. Low levels (≤4% TRR, ≤0.002 ppm) were also detected in grain of the following metabolites: CGA-265307, CGA-353968, CGA-355190, NOA-421275, NOA-407475, CGA-353042, NOA-405217, and CGA-382191.

Analysis of residual solids following solvent extraction indicated that radioactivity in fodder was also associated with pectins (≤0.5% TRR), lignins (1-7% TRR), and cellulose (≤1.4% TRR), and that radioactivity in grain was associated with proteins (3-4% TRR) and starch (7-10% TRR), suggesting that there is some incorporation of residues into natural plant constituents.

Table 12. Summary of the characterization/identification of ^{14}C-residues in corn forage, fodder, and grain grown from seed treated with [^{14}C]thiamethoxam at ~1.2 mg ai/plant (0.13 lb ai/A).

Metabolite/ fraction	[Thiazol -^{14}C]Thiamethoxam						[Oxadiazin-^{14}C]Thiamethoxam					
	Forage (0.113 ppm)		Fodder (0.346 ppm)		Grain (0.023 ppm)		Forage (0.104 ppm)		Fodder (0.238 ppm)		Grain (0.015 ppm)	
	%TRR [a]	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Thiamethoxam	8.1	0.009	4.3	0.015	6.5	0.002	7.9	0.008	3.0	0.007	15.1	0.002
CGA-322704	12.3	0.014	4.3	0.015	7.5	0.002	9.8	0.010	3.6	0.009	9.6	0.001
CGA-265307	2.8	0.003	1.0	0.003	-- [c]	--	1.9	0.002	0.5	0.001	--	--
CGA-355190	1.2	0.001	0.5	0.002	--	--	1.4	0.002	0.4	<0.001	--	--
NOA-407475	8.8	0.010	7.1	0.025	--	--	8.4	0.009	8.5	0.020	--	--
NOA-421275	12.2	0.014	10.4	0.036	--	--	11.7	0.012	10.1	0.024	--	--
CGA-353042	--	--	--	--	--	--	4.0	0.004	3.2	0.008	--	--
NOA-405217	--	--	--	--	--	--	2.6	0.003	1.0	0.002	--	--
CGA-382191	--	--	--	--	--	--	7.7	0.008	9.8	0.023	--	--
CGA-349208/CGA-330050	--	--	0.8	0.003	--	--	--	--	--	--	--	--
Total identified	**45.4**	**0.051**	**28.4**	**0.099**	**14.0**	**0.003**	**55.4**	**0.058**	**40.1**	**0.095**	**24.7**	**0.004**
TLC unknown(s) [d]	17.7	0.020	16.9	0.058	--	--	14.3	0.015	23.4	0.056	--	--
Unresolved ^{14}C-residue [e]	9.9	0.011	4.8	0.017	2.0	<0.001	4.4	0.005	5.2	0.012	2.5	<0.001
Aqueous fraction(s)	--	--	--	--	19.6	0.005	--	--	--	--	40.5	0.006
Organic fraction(s)	1.6	0.002	1.9	0.007	3.1	<0.001	2.8	0.003	3.5	0.008	3.5	<0.001
Total identified/ characterized	**74.6**	**0.084**	**52.0**	**0.181**	**38.7**	**0.009**	**76.9**	**0.081**	**72.2**	**0.172**	**71.2**	**0.011**
Residual solids	25.0	0.028	49.1	0.170	65.4	0.015	21.3	0.022	30.9	0.074	24.8	0.004

[a] %TRR not corrected for recovery.
[b] Expressed in [^{14}C]thiamethoxam equivalents.
[c] -- = Not detected.
[d] Specific unknowns isolated by TLC each accounted for ≤8.1% of the TRR.
[e] Radioactivity on TLC plates not associated with a specific region.

Table 13. Summary of the characterization/identification of ^{14}C-residues in corn forage, fodder, and grain grown from corn seedlings treated with [^{14}C]thiamethoxam as a soil drench at ~4.1 mg ai/plant (0.43 lb ai/A).

Metabolite/ fraction	[Thiazol -^{14}C]Thiamethoxam						[Oxadiazin-^{14}C]Thiamethoxam					
	Forage (0.402 ppm)		Fodder (0.880 ppm)		Grain (0.080 ppm)		Forage (0.349 ppm)		Fodder (1.030 ppm)		Grain (0.041 ppm)	
	%TRR [a]	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Thiamethoxam	27.7	0.111	5.3	0.047	7.9	0.006	28.1	0.098	3.1	0.032	15.1	0.006
CGA-322704	17.3	0.069	3.9 [c]	0.034	9.2 [f]	0.007	15.6	0.054	3.6	0.038	15.8	0.006
CGA-265307	1.7	0.007	0.5	0.004	1.4	0.001	1.0	0.004	0.5	0.005	2.2	0.001
CGA-355190	1.6	0.006	0.4	0.003	0.4	<0.001	1.7	0.006	0.3	0.004	--	--
CGA-353968	-- [d]	--	--	--	--	--	--	--	0.5	0.005	1.2	<0.001
NOA-421275	9.9	0.040	9.5	0.083	1.2	0.001	8.3	0.029	8.7	0.090	1.9	0.001
NOA-407475	8.6	0.035	7.7	0.068	0.5	<0.001	10.4	0.036	6.9	0.071	2.5	0.002
CGA-353042	--	--	--	--	--	--			3.8	0.040		
NOA-405217	--	--	--	--	--	--	2.1	0.008	0.8	0.008	4.1	0.002
CGA-382191	--	--	--	--	--	--	4.6	0.016	9.7	0.100	1.8	<0.001
CGA-349208/CGA-330050	--	--	0.8	0.007	0.4	<0.001	--	--	--	--	--	--
Total identified	**66.8**	**0.268**	**28.1**	**0.246**	**21.0**	**0.017**	**71.8**	**0.251**	**37.9**	**0.393**	**44.6**	**0.018**
TLC unknown(s) [e]	9.2	0.037	17.8	0.157	14.6	0.012	5.3	0.018	21.7	0.224	17.8	0.006
Unresolved ^{14}C-residue [f]	8.7	0.035	5.5	0.048	2.7	0.002	7.6	0.026	6.5	0.067	9.7	0.004
Organic fraction(s)	--	--	2.0	0.018	3.0	0.002	--	--	2.7	0.028	2.9	0.001
Total identified/ characterized	**84.7**	**0.340**	**53.4**	**0.469**	**41.3**	**0.033**	**84.7**	**0.295**	**68.8**	**0.712**	**75.0**	**0.029**
Residual solids	15.7	0.063	42.4 [g]	0.374	61.5 [g]	0.049	12.2	0.043	33.7 [g]	0.347	26.4 [g]	0.011

[a] %TRR not corrected for recovery.
[b] Expressed in [^{14}C]thiamethoxam equivalents.
[d] -- = Not detected.
[c] Fraction includes minor amounts of CGA-353968.
[e] Specific unknowns isolated by TLC each accounted for ≤8.1% of the TRR.
[f] Radioactivity on TLC plates not associated with a specific region.
[g] Fractionation of residual solids from fodder and grain are detailed in Tables 10 and 11, respectively.

Table 14. Summary of the characterization/identification of ^{14}C-residues in mature corn leaves harvested 78 days following a stem injection of [^{14}C]thiamethoxam at ~1.2 mg ai/plant.

Metabolite/ fraction	[Thiazol-^{14}C] Leaves (66.7 ppm)		[Oxadiazin-^{14}C] Leaves (59.1 ppm)	
	%TRR [a]	ppm [b]	%TRR	ppm
Thiamethoxam	48.4	32.28	51.9	30.67
CGA-322704	12.0	8.00	11.6	6.86
CGA-265307	1.3	0.87	2.2	1.30
CGA-355190	3.6	2.40	3.6	2.13
Desmethyl-CGA-353968	0.8	0.53	--	--
NOA-421275	1.3	0.87	1.6	0.95
NOA-407475	4.3	2.87	6.3	3.72
NOA-405217	--	--	1.6	0.95
CGA-382191	--	--	1.6	0.95
CGA-349208/CGA-330050	3.3	2.20	--	--
Total identified	**75.0** [d]	**50.02**	**80.4** [e]	**47.53**
TLC unknown(s) [f]	2.8	1.87	4.1	2.42
Unresolved ^{14}C-residue [g]	8.2	5.45	6.8	4.02
Total identified/ characterized	**86.0**	**57.34**	**91.3**	**53.97**
Residual solids	14.5	9.67	9.4	5.55

[a] %TRR not corrected for recovery.
[b] Expressed in [^{14}C]thiamethoxam equivalents.
[c] -- = Not detected.
[d] Trace amounts (<1% TRR) of CGA-353968, CGA-309335, and CGA-359683 were also identified.
[e] Trace amounts (<1% TRR) of CGA-353968 and desmethyl-CGA-353968 were also identified.
[f] Specific unknowns isolated by TLC each accounted for ≤2% of the TRR.
[g] Radioactivity on TLC plates not associated with a specific region.

Proposed Metabolic Pathway in Plants:

The metabolism of thiamethoxam in pears, cucumbers, and corn is similar, although the relative levels of individual metabolites differed among the three crops. To varying degrees, the metabolism of thiamethoxam in each of these crops involves: i) opening of the oxadiazine ring by hydrolysis, ii) loss of the nitro group, iii) hydrolysis of the guanidine moiety to urea derivatives, iv) cleavage of the N-C bridge between the two ring systems, and v) N-demethylation of the oxadiazine ring or its derivatives. Although the extract sequence of these reactions in individual crops is uncertain, metabolites resulting from each of these reactions were present in pears, cucumbers, and corn.

Initial hydrolysis the oxadiazine ring of thiamethoxam yields CGA-322704, which is a major metabolite in both pears and corn RACs. CGA-322704 can then either i) lose its nitro group to

form NOA-421275 (a major metabolite in corn), ii) undergo N-demethylation to yield CGA-265307, or iii) be cleaved at the N-C bridge to form NOA-405217 and thiazole ring metabolites (CGA-359683 and CGA-349208). Alternatively, thiamethoxam may initially lose its nitro group to form NOA-407475, which can then undergo hydrolysis of the oxadiazine ring to form NOA-421275, or hydrolysis of the imine to form CGA-355190.

In a meeting held on 7/28/99 (see memo of G.J. Herndon dated 8/31/99), the Metabolism Assessment Review Committee (MARC) concluded that the residue of concern for the proposed seed treatment use on canola is understood. The residue to be regulated (for risk assessment and tolerance setting purposes) is the parent thiamethoxam and its CGA-322704 metabolite.

In the MARC meeting held on 7/28/99, based on the low radioactivity present and identified in the cucumber metabolism study, the Committee recommended that another plant metabolism study be submitted to fulfill the requirement of 3 plant meatbolism studies for OPPTS 860.1300. Based on the uses requested under PP#9F5046 and 9F5051, the Committee recommended that a new plant metabolism study be conducted on a leafy vegetable (one of the representative commodities in either Crop Group 4 or 5).

RAB2 concludes that the two acceptable plant metabolism studies (pears and corn) would cover all proposed seed treatment (wheat, sorghum, barley, canola, cotton, tobacco, leafy (except Brassica) vegetables, Brassica leafy vegetables, cucurbit vegetables, and fruiting vegetables) uses (due to expected non-detectable residues), the proposed foliar treatment uses on cotton and canola (due to the edible portion (oil) undergoing many processing steps which would be expected to degrade or volatilize thiamethoxam residues), foliar uses on tobacco, foliar uses on fruiting (except cucurbit) vegetables (Crop Group 8), foliar uses on cucurbit vegetables (Crop Group 9), foliar and soil treatment of tuberous and corm vegetables (Crop Subgroup 1-C) (due to non-detectable residues in the field trials), and foliar uses on pome fruit (due to acceptable pear metabolism study).

The additional leafy vegetable plant metabolism will need to be conducted by the petitioner, reviewed and found acceptable by the Agency prior to the granting of the following proposed uses of thiamethoxam: foliar treatment of leafy (except Brassica) vegetables (Crop Group 4) and foliar treatment of Brassica leafy vegetables (Crop Group 5).

OPPTS GLN 860.1300: Nature of the Residue in Animals

Ruminants

With the current petition, Novartis has submitted two studies on the metabolism of [^{14}C]thiamethoxam by lactating goats following multiple oral doses. The in-life phases of these studies were conducted at Novartis Animal Health, Inc., Agricultural Research Center (CRA), St. Aubin, FR, Switzerland. The analytical phase was conducted at Novartis Crop Protection AG, Basel, Switzerland. Results from these studies are reported in:

44703510 Rumbeli, R. (1998) Metabolism of (Thiazol-2-^{14}C) CGA-293343 After Multiple Oral Administration to Lactating Goats: Lab Project Number: 027AM03: 992-98: CRA 96/089. Unpublished study prepared by Novartis Crop Protection AG. 269 p.

44703535 Lutringer, C. (1998) Metabolism of (Oxadiazin-4-^{14}C) CGA-293343 After Multiple Oral Administration to Lactating Goats: Lab Project Number: 027AM05: 1014-98. Unpublished study prepared by Novartis Crop Protection AG. 157 p.

The [thiazol-2-^{14}C]thiamethoxam had a specific activity of 16.2 μCi/mg and a radiochemical purity of 99.3%, and the [oxadiazin-4-^{14}C]thiamethoxam had a specific activity of 17.3 μCi/mg and a radiochemical purity of 96.2%. Two goats were dosed orally with each test substance via capsule at mean doses equivalent to 100.6 and 111.9 ppm in the diet, for [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]thiamethoxam, respectively. The nominal dose level of 100 ppm is equivalent to 70x the maximum theoretical dietary exposure of 1.43 ppm for dairy cattle, which is based on proposed tolerances (PP#9F5046) and a diet consisting of 60% wheat forage, 20% cotton gin byproducts, and 20% barley or wheat grain. Animals were dosed daily at 0, 24, 48, and 72 hours. Milk was collected twice daily and urine and feces were collected daily. The animals were sacrificed 6 hours after the last dose and blood, leg muscle, tenderloin, perirenal fat, omental fat, liver, kidneys, and bile were collected. Cage washes and debris were also collected. Samples were stored at ≤-18 C.

Total radioactive residues (TRR)

Radioactivity in liquid samples was determined by LSC directly. Solid samples were radioassayed after combustion (feces and fecal solids) or solubilization (tissues or non-extractables of tissues. The LOQs for the radioassays were 0.0003 ppm in milk and 0.0015-0.0027 ppm in tissues. For both ^{14}C-test substances, the dosed radioactivity was eliminated primarily in the urine (44-49%) and feces (8-12%), and ~1% was secreted in the milk. Radioactivity remaining in edible tissues at sacrifice accounted for 3.4-3.7% of the dose. Minor amounts of radioactivity (~0.6%) were detected in blood and bile and 18-26% was present in the gastrointestinal (GI) tract and rumen at sacrifice.

The TRR in milk and edible tissues are summarized in Table 15. Residues in milk plateaued within 31 hours in the [thiazol-^{14}C] study and within 55 hours in the [oxadiazin-^{14}C] study; maximum milk residues were 1.9 and 2.3 ppm, respectively. TRR in tissues observed in the two studies were comparable. Residue levels were highest in liver (~11 ppm) and kidney (6.6-7.5 ppm). Fat contained the lowest concentration of radioactivity (0.26-0.65 ppm) and muscle contained 2.0-2.3 ppm.

Table 15. Total radioactive residues in milk and edible tissues of goats dosed with [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at ~100 ppm for 3 days.

Matrix	Interval (hours)	Total Radioactive Residues (ppm) [a]					
		[Thiazol-2-^{14}C]			[Oxadiazin-4-^{14}C]		
		Goat 1	Goat 2	Mean	Goat 1	Goat 2	Mean
Milk	0-7	1.606	1.358	1.483	1.592	1.733	1.670
	7-24	1.019	0.436	0.726	0.589	1.122	0.866
	24-31	2.191	1.618	1.904	1.806	2.326	2.096
	31-48	0.543	1.181	0.868	0.747	1.331	1.045
	48-55	2.041	1.712	1.872	1.742	2.658	2.308
	55-72	1.193	0.519	0.793	1.532	1.519	1.522
	72-78	1.953	1.504	1.688	2.167	2.316	2.280
	Total	1.322	1.039	1.173	1.165	1.703	1.481
Leg muscle	78	2.453	1.704	2.081	2.069	2.521	2.267
Tenderloin muscle	78	2.341	1.721	2.038	2.038	2.526	2.275
Omental fat	78	0.206	0.269	0.257	0.320	0.997	0.458
Perirenal fat	78	0.653	0.558	0.579	0.376	1.860	0.648
Kidney	78	7.839	5.611	6.633	6.588	8.382	7.520
Liver	78	12.11	9.855	11.09	10.14	11.77	11.04

[a] Expressed in [^{14}C]thiamethoxam equivalents.

Extraction of residues

Tissues and milk from all collections from the two goats in each group were pooled prior extraction. Leg and tenderloin muscle were pooled and omental and perirenal fat were composited. ^{14}C-Residues in milk, feces, and tissues were extracted sequentially with ACN and ACN:water (8:2, v/v); additionally radioactivity in muscle (oxadiazin-^{14}C), liver, and kidney was also extracted with MeOH:water (8:2, v/v). Solvent extracts were pooled by matrix and extracted ^{14}C-residues from milk (oxadiazin-^{14}C), muscle (thiazol-^{14}C) and fat were concentrated and partitioned between ACN and hexane. Extracts or solvent fractions from milk, muscle, fat, liver (oxadiazin-^{14}C), and kidney (oxadiazin-^{14}C) were analyzed directly by TLC and HPLC. ^{14}C-Residues in extracts from liver (thiazol-^{14}C) and kidney (thiazol-^{14}C) were purified by C_{18} column chromatography prior to analysis. The extraction and fractionation of ^{14}C-residues from milk and tissues are summarized in Table 16.

Non-extracted residues in muscle (oxadiazin-^{14}C), liver, and kidney were subjected to microwave-assisted extraction in 2-propanol:water (80:20, v/v) for a total of 40 minutes at sequentially higher temperatures of 130, 150, and 180 C. Residues thus solubilized from the non-extractable muscle fraction were purified by C_{18} solid phase extraction. In all matrices except liver, >90% of the TRR was extracted with ACN/water. In liver labeled with [thiazol-^{14}C]

and [oxadiazin-^{14}C]thiamethoxam, respectively, 15 and 31% of the TRR was unextracted initially; an additional 14 and 17% was solubilized by microwave-assisted extraction.

Characterization and identification of residues

In addition to the parent compound, 20 reference standards were used for metabolite identification. Extracted residues were characterized and quantified by co-chromatography with standard compounds using the three following 2D-TLC systems:

SS I	ethyl methyl ketone:MeOH:water:formic acid, 80:18:1:1
	ethyl acetate:2-propanol:water:formic acid, 85:13:1:1
SS II	ethyl methyl ketone:MeOH:water:formic acid, 60:38:1:1
	tetrahydrofuran:MeOH:water:formic acid, 60:35:4:1
SS III	tetrahydrofuran:MeOH:water:formic acid, 60:35:4:1
	chloroform:MeOH:water:formic acid, 75:20:2:4

In addition, residues were analyzed by HPLC comparing retention times with metabolite standards, using a C_{18} column with a gradient of 0.01 M sodium phosphate buffer (pH 7) or 0.01 M ammonium formate buffer (pH 5) to MeOH.

For definitive identification metabolites were isolated in a parallel work-up that involved solvent extraction as described above and subsequent preparative TLC and HPLC procedures. Each component of the radioactive residue was characterized by HPLC and at least one 2D-TLC system. The LOQs for metabolite identification were 0.006-0.064 ppm for liver, 0.005-0.055 ppm for kidney, 0.0005-0.016 ppm for muscle, 0.001-0.007 ppm for fat, and 0.007-0.011 ppm for milk. The following compounds' structures were confirmed by LC-MS: L14, CGA-355190, NOA-407475, NOA-421276, NOA-421275, and CGA-309335 from liver; and L14, CGA-355190, CGA-353968, NOA-421276, NOA-421275, desmethyl-CGA-353968, N1, MU12, and the parent compound from kidney.

Table 16. Distribution of radioactivity following extraction of residues from milk and tissues of goats dosed with [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at ~100 ppm for 3 days.

[thiazol-2-^{14}C]										
Fraction	Milk 1.173 ppm [a]		Muscle 2.078 ppm [a]		Kidney 6.633 ppm [a]		Liver 11.09 ppm [a]		Fat 0.389 [a]	
	% TRR	ppm [b]	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
ACN/water	98.1	1.151	94.7	1.968	91.2	6.049	84.8	9.404	93.4	0.363
Aqueous	NA [c]	NA	NA	NA	8.7	0.577	NA	NA	NA	NA
MeOH	NA	NA	NA	NA	82.5	5.472	NA	NA	NA	NA
Hexane	NA	NA	10.8	0.224	NA	NA	NA	NA	11.3	0.044
ACN	NA	NA	83.9	1.743	NA	NA	NA	NA	82.1	0.319
Non-extracted	1.9	0.022	5.3	0.110	8.8	0.584	15.2	1.686	6.6	0.026
microwave	NA	NA	NA	NA	8.6	0.570	13.9	1.542	NA	NA
non-extracted	NA	NA	NA	NA	0.2	0.013	1.3	0.144	NA	NA
[oxadiazin-4-^{14}C]										
Fraction	Milk 1.481 ppm		Muscle 2.267 ppm		Kidney 7.520 ppm		Liver 11.04 ppm		Fat 0.535 ppm	
	% TRR	ppm [b]	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
ACN/water	99.1	1.468	91.2	2.068	91.1	6.851	69.3	7.651	91.9	0.492
MeOH/water	NA	NA	2.8	0.063	NA	NA	NA	NA	NA	NA
Hexane	0.1	0.001	NA	NA	NA	NA	NA	NA	1.4	0.007
Aqueous	99.0	1.466	NA	NA	NA	NA	NA	NA	90.5	0.484
Non-extracted	0.9	0.013	6.0	0.136	8.9	0.669	30.7	3.389	8.1	0.043
microwave	NA	NA	2.6	0.058	6.0	0.451	17.3	1.910	NA	NA
non-extracted	NA	NA	3.4	0.077	2.9	0.218	13.4	1.479	NA	NA

[a] TRR are for composite samples from the two goats in each group: milk samples from all milkings were pooled; tenderloin and leg muscle were combined; perirenal and omental fat were combined.

[b] The concentrations (ppm) of radioactivity in each fraction was calculated by the reviewer from the TRR and percent TRR provided by the study author.

[c] NA = Not applicable; fraction not obtained from this matrix.

The components of the radioactive residue in milk and tissues from goats dosed with [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]thiamethoxam are summarized in Tables 17 and 18, respectively. The metabolic patterns of the two [^{14}C]thiamethoxam test substances were qualitatively and quantitatively similar. In milk, the thiamethoxam and CGA-322704 were the major residues, accounting for 31-37% TRR (0.362-0.545 ppm) and 44-45% TRR (0.514-0.661 ppm), respectively. Metabolite CGA-265307 accounted for 10-18% TRR (0.148-0.208 ppm), and desmethyl-CGA-353968 was a minor metabolite at 1.7-2.8% TRR. Metabolite NOA-405217, formed from cleavage of the oxadiazin moiety from the primary metabolite CGA-322704, also accounted for 2.8% of the TRR from the oxadiazin-label.

The metabolism of [^{14}C]thiamethoxam in liver was extensive; the parent was detected at ≤1% TRR. The major residues identified in liver included: NOA-421276 (8-22% TRR), NOA-407475 (9-11% TRR), NOA-421275 (11-13% TRR), and metabolite L-14 (13-25% TRR). Metabolite CGA-322704 was negligible in the initial liver extracts, but was released by microwave-assisted extraction at 6-7% TRR (0.701 and 0.799 ppm). Minor amounts of other metabolites were also released by microwave extraction.

In kidney, the parent compound was the predominant residue component from both labels, accounting for 21-22%TRR (1.40-1.68 ppm). NOA-421275 accounted for 16-20 % TRR in both studies; NOA-421276 accounted for 13% TRR in the thiazole study, and N-5 comprised 12% TRR in the oxadiazine study. Up to 10 additional metabolites were detected in minor amounts. In addition to the initial extraction, microwave-assisted extraction released small amounts of several metabolites.

The major residue in muscle and fat was thiamethoxam, accounting for 51-54% (1.1 and 1.2 ppm) and 36-52% TRR (0.138-0.278 ppm), respectively. CGA-322704 comprised 8-12% TRR in fat (0.041-0.048 ppm). NOA-421276 and NOA-421275 were also major metabolites in fat at 11-23% TRR. NOA-421276 accounted for 15% TRR in thiazol-labeled muscle and MU-12 comprised 11% TRR in the oxadiazine study. Five to seven additional minor metabolites were found in muscle and fat.

Urine and feces

Urine was analyzed directly by TLC (SSI) and HPLC. Residues in feces were extracted with ACN and ACN/water. The parent compound, CGA-322704, NOA-421275, and CGA-265307 were the major residues eliminated. The authors noted that the metabolic pathways in the goat, hen, and rat are essentially the same.

Storage stability:

Milk and tissue samples were stored at ≤-18 C for 11-14 months prior to definitive analysis for the [thiazol-^{14}C] study and up to 7 months prior to analysis for the [oxadiazin-^{14}C] study. To demonstrate the stability of ^{14}C-residues during the [thiazol-^{14}C] study, the petitioner conducted

an initial extraction of milk and liver subsamples within 1 month of collection and analyzed the extracts by TLC within 3 days. Results of these analyses were compared to a reanalysis of the initial extracts after 11 months of storage and to analysis of fresh extracts obtained from a subsample of milk extracted after 11 of storage and subsamples of liver extracted after 11 and 22 months of the storage. Similar data were generated to support the [oxadiazin-^{14}C] study. TLC analyses of milk and liver extracts obtained within 11 days of collection were compared to reanalyses of the same extracts 10 months later and to analyses of fresh extracts of milk and liver that had also been stored for 10 months. The metabolite TLC patterns were similar for each of these analyses in both studies, indicating that ^{14}C-residues were stable in stored milk and tissue samples and extracts for the duration of the studies. No additional storage stability data are required.

Conclusions: The studies of [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]thiamethoxam metabolism in goats are adequate. More than 74% of the radioactivity in milk and tissues was identified. The major pathway of thiamethoxam in ruminants involves cleavage of the oxadiazin ring to form CGA-322704 and subsequent demethylation to produce CGA-265307; loss of the nitro group from these two metabolites yields NOA-421275 and NOA-421276. The liver and kidney metabolites N5 and L14 are, respectively, acetic acid and 2-oxo-propionic acid conjugates formed from the hydrazide of the parent and MU12 is produced from L14 following cleavage of the oxadiazin ring. All of the aforementioned metabolites are bridge-intact; separation of the thiazole and oxadiazine moieties is only a minor pathway as evidenced by the low levels (<3% TRR) of label-specific metabolites such as CGA-359683 and NOA-405217.

Table 17. Characterization and identification of ^{14}C-residues in milk and tissues from goats dosed with [thiazol-2-^{14}C]thiamethoxam at ~100 ppm for 3 days.

Component	Milk (1.173 ppm)		Liver [a] (11.09 ppm)		Kidney [a] (6.633 ppm)		Muscle (2.078 ppm)		Fat (0.389 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Thiamethoxam	30.8	0.362	1.0	0.107	19.8 *21.1*	1.311 *1.400*	51.4	1.068	35.5	0.138
CGA-322704	43.8	0.514	0.6 *7.2*	0.062 *0.799*	2.0 *2.6*	0.130 *0.175*	9.4	0.196	12.2	0.048
NOA-407475	ND [b]	ND	10.7	1.191	2.4	0.159	0.5	0.011	ND	ND
CGA-265307	17.7	0.208	2.2 *3.8*	0.249 *0.424*	0.2 *0.9*	0.012 *0.058*	3.2	0.066	3.1	0.012
NOA-404617	ND	ND	0.2	0.027	4.1	0.271	ND	ND	ND	ND
NOA-421275	ND	ND	10.1 *13.2*	1.120 *1.466*	17.5 *19.8*	1.163 *1.315*	5.6	0.117	10.9	0.043
NOA-421276	ND	ND	20.4 *22.3*	2.257 *2.470*	10.9 *13.2*	0.723 *0.877*	14.6	0.303	23.3	0.908
L-14	ND	ND	13.1	1.449	9.8	0.649	ND	ND	ND	ND
MU-12	ND	ND	5.9	0.658	9.3	0.614	6.6	0.137	4.6	0.018
Desmethyl-CGA-353968	2.8	0.033	1.4	0.151	1.4	0.095	2.9	0.060	2.7	0.011
CGA-355190	ND	ND	2.6	0.284	2.0	0.136	ND	ND	ND	ND
CGA-353968	ND	ND	1.3	0.142	1.9	0.124	ND	ND	ND	ND
CGA-309335	ND	ND	2.7	0.302	ND	ND	ND	ND	ND	ND
CGA-359683	ND	ND	0.6	0.062	1.5	0.102	ND	ND	ND	ND
Total identified	95.2	1.117	72.7 *86.0*	8.061 *9.532*	82.7 *90.1*	5.488 *5.975*	94.2	1.957	92.5	0.360
Polar	2.9	0.034	8.9	0.987	3.7	0.243	ND	ND	ND	ND
Non-polar	ND	ND	3.2 *3.8*	0.356 *0.426*	4.8 *6.1*	0.319 0.402	0.5	0.011	0.9	0.004
Non-extracted	1.9	0.022	15.2 *1.3*	1.686 *0.144*	8.8 *0.2*	0.584 *0.013*	5.3	0.110	6.6	0.026

[a] Values in *italics* include residue components released from non-extractable fractions by microwave-assisted extraction.

[b] ND = not quantifiable.

Table 18. Characterization and identification of ^{14}C-residues in milk and tissues from goats dosed with [oxadiazin-4-^{14}C]thiamethoxam at ~100 ppm.

Component	Milk (1.481 ppm)		Liver [a] (11.04 ppm)		Kidney [a] (7.520 ppm)		Muscle (2.267 ppm)		Fat (0.535 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Thiamethoxam	36.8	0.545	ND [b] *1.1*	ND *0.120*	22.3	1.679	53.6	1.215	51.9	0.278
CGA-322704	44.6	0.661	ND *6.4*	ND *0.701*	ND	ND	4.5	0.102	7.6	0.041
NOA-407475	ND	ND	9.2	1.016	5.3	0.400	1.5	0.034	ND	ND
CGA-265307	10.0	0.148	0.6 *2.2*	0.068 *0.241*	ND	ND	2.1	0.047	1.6	0.009
NOA-404617	ND	ND	ND	ND	ND	ND	ND	ND	0.2	0.001
NOA-421275	ND	ND	9.7 *11.1*	1.072 *1.221*	15.7 *16.4*	1.178 *1.235*	4.6	0.105	13.3	0.071
NOA-421276	ND	ND	4.9 *8.1*	0.540 *0.890*	4.8 *6.3*	0.362 *0.477*	5.0	0.113	13.2	0.071
L-14	ND	ND	23.0 *25.1*	2.545 *2.776*	8.5 *8.9*	0.636 *0.669*	5.6	0.126	ND	ND
MU-12	ND	ND	5.3	0.581	7.5 *7.8*	0.561 *0.588*	10.9	0.247	ND	ND
Desmethyl-CGA-353968	1.7	0.025	0.7	0.073	1.0	0.072	1.2	0.027	1.0	0.005
CGA-355190	ND	ND	1.3	0.140	2.5	0.191	ND	ND	ND	ND
N-5	ND	ND	3.6	0.401	11.8	0.887	ND	ND	ND	ND
NOA-405217	2.8	0.042	0.5	0.055	1.6	0.118	1.4	0.032	1.7	0.009
Total identified	96.0	1.421	58.8 *74.4*	6.492 *8.216*	80.9 *84.0*	6.085 *6.316*	90.3	2.048	90.5	0.484
Polar	2.2	0.032	3.6 *5.0*	0.400 *0.547*	7.6 *8.9*	0.568 *0.668*	1.7	0.037	ND	ND
Non-polar	1.0	0.014	6.9 *7.2*	0.757 *0.798*	2.6 *4.2*	0.199 *0.318*	2.0	0.045	1.4	0.007
Non-extracted	0.9	0.014	30.7 *13.4*	3.391 *1.480*	8.9 *2.9*	0.668 *0.218*	6.0	0.136	8.1	0.043

[a] Values in *italics* include residue components released from non-extractable fractions by microwave-assisted extraction.

[b] ND = not quantifiable.

Poultry

With the current petition, Novartis has submitted two studies on the metabolism of [^{14}C]thiamethoxam by hens following multiple oral doses. The in-life phases of these studies were conducted at Novartis Animal Health, Inc. Agricultural Research Center, Aubin, FR, Switzerland. The analytical phase was conducted at Novartis Crop Protection AG, Basel, Switzerland. Results from these studies are reported in:

44703526 Rumbeli, R. (1998) Metabolism of (Thiazol-2-^{14}C) CGA-293343 after Multiple Oral Administration to Laying Hens: Lab Project Number: 027AM04: 961-98: CRA 96/090. Unpublished study prepared by Novartis Crop Protection AG. 118 p.

44715115 Lutringer, C. (1998) Metabolism of (Oxadiazin-4-^{14}C) CGA-293343 after Multiple Oral Administration to Laying Hens: Lab Project Number: 1011-98: 027AM06: CRA 97/103. Unpublished study prepared by Novartis Crop Protection, Inc. 104 p.

The [thiazol-2-^{14}C]thiamethoxam had a specific activity of 16.2 μCi/mg and a radiochemical purity of 99.3%, and the [oxadiazin-4-^{14}C]thiamethoxam had a specific activity of 17.3 μCi/mg and a radiochemical purity of 96.2%. Two groups of five hens were dosed orally once daily with [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at zero, 24, 48, and 72 hours via capsule at mean doses of 111.7 and 97.6 ppm per day, respectively. The nominal dose level of 100 ppm is equivalent to ~4,000x the maximum theoretical dietary exposure of 0.026 ppm for poultry, which is based on proposed tolerances (PP#9F5046) and a diet consisting of 20% cotton seed meal and 80% sorghum grain.

Eggs were collected twice daily and excreta was collected daily. The animals were sacrificed 6 hours after the last dose, and blood, thigh and breast muscle, peritoneal fat, skin with attached fat, liver, kidneys, bile and GI tract contents were collected. Cage washes and debris were also collected. Samples were stored a ≤-18 C until analysis.

Total radioactive residues (TRR)

Radioactivity in liquid samples was determined by LSC directly. Solid samples were radioassayed after combustion (excreta and gastrointestinal material) or solubilization (tissues or non-extractables of tissues). The LOQs for the radioassays were 0.0007-0.0027 ppm for eggs and tissues. Of the total radioactive dose, ~80% was excreted and ~0.1% was secreted in the eggs. Radioactivity remaining in edible tissues accounted for 1.3-1.5% of the dose.

The TRR in eggs and edible tissues are summarized in Table 19. Residues in egg whites reached maximum levels within 48 hours (0.357 ppm) in the thiazol-^{14}C study and within 72 hours (0.412 ppm) in the oxadiazin-^{14}C study. The highest residue concentrations in egg yolks was observed in the last collection interval (72-78 hours) at 0.573 and 0.565 ppm. As seen in goats, liver and kidney had the highest TRR at 8-9 ppm and 4.4-5.2 ppm, respectively.

Table 19. Total radioactive residues in eggs and edible tissues of hens dosed for 4 days with [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at ~100 ppm/day.

Matrix	Sampling Interval (hours)	Total Radioactive Residues (ppm) [a]	
		[Thiazol-2-^{14}C]	[Oxadiazin-4-^{14}C]
Egg white	0-24	0.046	0.028
	24-48	0.357	0.320
	48-72	0.330	0.412
	72-78	0.331	0.402
	0-78	0.265	0.292
Egg yolk	0-24	0.006	0.011
	24-48	0.227	0.193
	48-72	0.350	0.399
	72-78	0.573	0.565
	0-78	0.290	0.295
Whole egg	0-78	0.272	0.293
Muscle	78	0.677	0.929
Skin/attached fat	78	0.307	0.408
Peritoneal fat	78	0.233	0.177
Total skin/fat	78	0.290	0.366
Kidney	78	4.435	5.192
Liver	78	8.017	9.145

[a] Expressed in [^{14}C]thiamethoxam equivalents; data are the average of five hens per dose group.

Extraction of residues

Egg white and egg yolk each were pooled from all collection intervals from the five hens in each group prior extraction. Thigh and breast muscle were pooled by group. Aliquots of skin/fat and peritoneal fat were combined.

In the thiazol-label study, ^{14}C-residues in each matrix were extracted sequentially with ACN and ACN:water (8:2, v/v), and the extracts were combined. The extracted residues from egg whites were directly analyzed by TLC and HPLC, and the extracted residues from skin/fat were partition with hexane and then analyzed. Residues in extracts from egg yolk, muscle, and liver were further fractionated using a C_{18} SPE C_{18} column eluted as follows:

egg yolk - 0.035 M phosphate buffer pH 7, MeOH/water, MeOH, hexane
muscle - 0.035 M phosphate buffer pH 7, MeOH/water, MeOH
liver - 0.02 M phosphate buffer pH 6, MeOH/water, MeOH, MeOH/THF

Residues in eluant fractions were then analyzed by TLC and HPLC.

In the [oxadiazin-^{14}C] study, ^{14}C-residues were extracted sequentially with ACN and ACN:water (8:2, v/v), and the extracts were combined. The extract from egg whites was analyzed directly by TLC and HPLC. Extracted ^{14}C-residues from egg yolk, muscle, skin/fat, and liver were partitioned with hexane. The hexane fractions from each matrix and the ACN:water fractions from yolks and muscle were then analyzed by TLC and HPLC. The ACN:water fractions from liver and skin/fat were first fractionated using a C_{18} SPE column eluted with water and MeOH, and the resulting water fractions were further cleaned up by SPE, prior to TLC and HPLC analysis.

In both studies, non-extracted residues in muscle and liver were subjected to microwave-assisted extraction in 2-propanol:water (80:20, v/v) for a total of 40 minutes at sequential temperatures of 130, 150, and 180 C. Residues thus solubilized from the non-extractable muscle fraction were purified by C_{18} SPE, eluted with pH 7 phosphate buffer and MeOH.

The distribution of radioactivity following the extraction of residues from eggs and poultry tissues are presented in Table 20. With the exception of liver from both studies and muscle from the [thiazol^{14}C] study, >90% of the TRR in egg white and tissues were extracted with ACN/water. Unextracted ^{14}C-residues initially accounted for 49-50% TRR in liver and 9-11% TRR in muscle; microwave-assisted extraction solubilized an additional 44-49% TRR from liver and 6.0-11% TRR from muscle.

Table 20. Distribution of radioactivity following extraction of residues from eggs and tissues of hens receiving 4 doses of [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at ~100 ppm over a period of 72 hours.

[thiazol-2-^{14}C]										
Fraction	Egg white (0.265 ppm)[a]		Egg yolk (0.290ppm)		Muscle (0.677 ppm)		Liver (8.017 ppm)		Skin/fat (0.290 ppm)	
	% TRR	ppm [b]	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
ACN/water	98.2	0.260	98.1	0.284	88.8	0.601	49.7	3.984	97.0	0.281
Hexane	NA[c]	NA	NA	NA	NA	NA	NA	NA	3.0	0.009
ACN	NA	NA	NA	NA	NA	NA	NA	NA	94.0	0.273
SPE buffer	NA	NA	1.7	0.005	29.4	0.199	1.5	0.120	NA	NA
SPE MeOH/water	NA	NA	95.0	0.276	59.4	0.402	47.7	3.824	NA	NA
SPE other	NA	NA	1.4	0.004	NA	NA	0.5	0.040	NA	NA
Non-extracted	1.8	0.005	1.9	0.006	11.2	0.076	50.3	4.033	3.0	0.009
microwave	NA	NA	NA	NA	11.1	0.075	49.3	3.952	NA	NA
non-extracted	NA	NA	NA	NA	0.1	<0.001	1.0	0.080	NA	NA
[oxadiazin-4-^{14}C]										
Fraction	Egg white (0.292 ppm)		Egg yolk (0.295 ppm)		Muscle (0.929 ppm)		Liver (9.145 ppm)		Skin/fat (0.366 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
ACN/water	98.6	0.288	94.8	0.280	91.3	0.848	50.8	4.646	93.2	0.341
Hexane	NA	NA	0.4	0.001	12.0	0.111	0.7	0.064	2.0	0.007
Aqueous	NA	NA	94.3	0.278	79.2	0.736	50.1	4.582	91.2	0.334
SPE water	NA	NA	NA	NA	NA	NA	16.1	1.472	9.7	0.036
SPE MeOH	NA	NA	NA	NA	NA	NA	33.9	3.100	81.5	0.298
Non-extracted	1.4	0.004	5.2	0.015	8.7	0.081	49.2	4.499	6.9	0.025
microwave	NA	NA	NA	NA	6.0	0.056	44.0	4.024	NA	NA
non-extracted	NA	NA	NA	NA	2.8	0.026	5.2	0.476	NA	NA

[a] TRR are for composite samples from the five hens in each group: egg yolk and white samples were pooled from all collections; breast and thigh muscle were combined; peritoneal fat and skin with attached fat were combined.

[b] The concentrations (ppm) of radioactivity in each fraction was calculated by the reviewer from the TRR and percent TRR provided by the study author.

[c] NA = Not applicable; fraction not obtained from this matrix.

Characterization and Identification of residues

Extracted residues were characterized and quantified by co-chromatography with standard compounds using the three following 2D-TLC systems:

SS I	ethyl methyl ketone:MeOH:water:formic acid, 80:18:1:1
	ethyl acetate:2-propanol:water:formic acid, 85:13:1:1
SS II	ethyl methyl ketone:MeOH:water:formic acid, 60:38:1:1
	tetrahydrofuran:MeOH:water:formic acid, 60:35:4:1
SS III	tetrahydrofuran:MeOH:water:formic acid, 60:35:4:1
	chloroform:MeOH:water:formic acid, 75:20:2:4

In addition, residues were analyzed by HPLC comparing retention times with metabolite standards, using a C_{18} column with a gradient of 0.01 M sodium phosphate buffer (pH 7) or 0.01 M ammonium formate buffer (pH 5) to MeOH.

Each component of the radioactive residue was characterized by HPLC and at least one 2D-TLC system. The LOQs for metabolite quantification were 0.013-0.052 ppm in liver, ≤0.010 ppm in egg white, and ≤0.005 ppm for the other matrices. For definitive identification, metabolite MU3 was isolated from thiazol-labeled muscle in a parallel work-up that involved solvent extraction as described above and subsequent preparative TLC and HPLC procedures. The structure of MU3 was confirmed by LC/MS.

The components of the radioactive residue in eggs and tissues from hens dosed with [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]thiamethoxam are summarized in Tables 21and 22, respectively. For a given matrix, the major metabolites were essentially the same in both studies. Slight differences in minor metabolite patterns may be attributable to the use of different extraction and partitioning procedures in the two studies.

The principal ^{14}C-residues in eggs were CGA-265307, accounting for 45-47% TRR in whites and 54-59% TRR in yolks, and CGA-322704 accounting for 20-25% TRR in whites and yolks. Thiamethoxam was also detected in whites at 2-5% TRR and in yolks at 11% TRR. In egg whites, NOA-4046517 (9-15% TRR) also accounted for a substantial portion of the residue.

In liver from the two studies, the major residues were CGA-322704 (34-39% TRR), CGA-265307 (16-20% TRR), and MU3 (12-22% TRR). In the thiazol-label study, NOA-421275 was also detected at 13% TRR. Thiamethoxam was detected in only trace amounts (0.2% TRR) in liver. Most of the CGA-322704, >30% TRR (~2.5-3 ppm), was in the fractions solubilized by microwave-assisted extraction. Microwave extraction also released additional amounts of CGA-265307 and NOA-421275.

In muscle, the major residues included MU3 (28-39% TRR) and thiamethoxam (21% TRR). In the thiazol-^{14}C] study, NOA-421275 also comprised 11% TRR in muscle. In skin/fat, the major

residue was CGA-265307, accounting for 54-58% TRR, with thiamethoxam and CGA-322704 accounted for 5-15% TRR and 8-9% TRR, respectively.

Storage stability

Egg and tissue samples were stored at -18 C for 13-18 months prior to the definitive analyses in the [thiazol-^{14}C] study and for up to 6 months in the [oxadiazin-^{14}C] study. To demonstrate the stability of ^{14}C-residues during the [thiazol-^{14}C] study, the petitioner conducted an initial extraction of egg whites and liver subsamples within 1 month of collection and analyzed the extracts by TLC within 3 days. Results of these analyses were compared to a reanalysis of the initial extracts after 11 months of storage and to analysis of fresh extracts obtained from a subsamples of egg whites and liver extracted after 11 and 22 months of the storage. Similar data were generated to support the [oxadiazin-^{14}C] study. TLC analyses of egg whites and liver extracts obtained within 9 days of collection were compared to reanalyses of the same extracts 6 months later and to analyses of fresh extracts of egg whites and liver that had also been stored for 6.6 months. The metabolite TLC patterns were similar for each of these analyses in both studies, indicating that ^{14}C-residues were stable in stored egg and tissue samples and extracts for the duration of the studies. No additional storage stability data are required.

Conclusions: The studies of [thiazol-2-^{14}C] and [oxadiazin-4-^{14}C]thiamethoxam metabolism in hens are adequate. More than 67% of the radioactivity in eggs and tissues was identified. Thiamethoxam is metabolized in poultry primarily either by (I) cleavage of the oxadiazine ring to form CGA-322704 and subsequent demethylation to produce CGA-265307, or (ii) loss of the nitro group from the parent molecule and oxadiazine ring cleavage to form NOA-421275. The major muscle and liver metabolite MU3 is an acetic acid conjugate of CGA-265307. Separation of the thiazole and oxadiazine rings is a minor pathway; the label specific metabolites NOA-402988 (thiazole) and NOA-405217 (oxadiazine) each accounted for $\leq$1.4% TRR in any matrix.

Table 21. Characterization and identification of ^{14}C-residues in eggs and tissues from hens dosed with [thiazol-2-^{14}C]thiamethoxam at ~100 ppm.

Component	Egg white (0.265 ppm)		Egg yolk (0.290 ppm)		Liver [a] (8.017 ppm)		Muscle (0.677 ppm)		Skin/Fat (0.290 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Thiamethoxam	5.0	0.013	11.3	0.033	ND	ND	21.1	0.143	14.8	0.043
CGA-322704	24.8	0.066	23.2	0.067	3.2 *34.0*	0.257 *2.722*	3.2	0.022	9.2	0.027
CGA-265307	45.0	0.119	58.9	0.171	14.9 *19.9*	1.198 *1.599*	7.0	0.047	54.2	0.157
NOA-421275	ND	ND	ND	ND	3.3 *12.7*	0.265 *1.015*	10.7	0.073	3.4	0.010
NOA-404617	8.6	0.023	ND	ND	ND	ND	ND	ND	1.8	0.005
MU3	ND	ND	ND	ND	21.9	1.758	38.7	0.262	8.3	0.024
Desmethyl-CGA-353968	2.4	0.006	ND	ND	1.2	0.094	4.8	0.032	3.0	0.009
NOA-402988	ND	ND	ND	ND	1.3	0.103	ND	ND	ND	ND
Total identified	85.8	0.277	93.4	0.271	45.8 *90.9*	3.671 *7.287*	85.4	0.578	94.7	0.275
Polar	8.8	0.023	3.3	0.010	1.5 *2.8*	0.120 *0.228*	1.3	0.009	2.3	0.007
Non-polar	3.6	0.010	1.4	0.004	0.5 *3.3*	0.040 *0.269*	2.1	0.015	ND	ND
Non-extracted	1.8	0.005	1.9	0.006	50.3 *1.0*	4.033 *0.009*	11.2	0.076	3.0	0.009

[a] Values in *italics* include residue components released from non-extractable fractions by microwave-assisted extraction.

Table 22. Characterization and identification of ^{14}C-residues in eggs and tissues from hens dosed with [oxadiazin-^{14}C]thiamethoxam at ~100 ppm.

Component	Egg white (0.292 ppm)		Egg yolk (0.295 ppm)		Liver [a] (9.145 ppm)		Muscle (0.929 ppm)		Skin/Fat (0.366 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Thiamethoxam	1.9	0.006	10.9	0.032	0.2	0.022	20.7	0.192	5.0	0.018
CGA-322704	20.4	0.060	19.5	0.058	2.8 *38.5*	0.253 *3.517*	1.5	0.014	7.7	0.028
NOA-407475	ND	ND	6.1	0.018	ND	ND	0.8	0.008	0.3	0.001
CGA-265307	47.2	0.138	53.7	0.159	12.0 *16.3*	1.100 *1.494*	8.4	0.078	57.4	0.210
NOA-405217	1.2	0.004	0.7	0.002	0.2 *0.4*	0.018 *0.040*	1.0	0.010	1.4	0.005
NOA-404617	14.6	0.043	ND	ND	0.2 *0.8*	0.020 *0.073*	ND	ND	ND	ND
NOA-421275	ND	ND	1.3	0.004	1.2	0.110	1.9	0.018	1.4	0.005
CGA-355190	4.2	0.012	ND	ND	ND	ND	2.4	0.023	5.6	0.021
MU3	ND	ND	ND	ND	12.0	1.093	28.1	0.261	3.6	0.013
8U	1.9	0.006	0.9	0.003	1.0	0.091	3.0	0.028	4.5	0.016
Total identified	91.4	0.267	93.1	0.275	29.6 *70.4*	2.708 ***6.441***	67.9	0.631	87.0	0.319
Polar	4.0	0.012	0.8	0.003	18.9 *20.4*	1.725 *1.869*	15.3	0.142	2.3	0.008
Non-polar	3.2	0.009	0.8	0.002	2.0 *3.6*	0.185 *0.330*	8.1	0.075	3.8	0.014
Non-extracted	1.4	0.004	5.2	0.016	49.2 *5.2*	4.499 *0.477*	8.7	0.081	6.8	0.025

[a] Values in *italics* include residue components released from non-extractable fractions by microwave-assisted extraction.

Overall Animal Metabolism Conclusions

The metabolism of thiamethoxam in ruminants and poultry is similar. The major pathway of metabolism involves hydrolysis of the oxadiazine ring to form CGA-322704 and subsequent demethylation to produce CGA-265307; loss of the nitro group from these two metabolites also yields NOA-421275 and NOA-421276. Several major metabolites (MU3, L14, and MU12) in both ruminants and poultry also result from the reduction of the nitro group in thiamethoxam or CGA-265307 to a hydrazine, and subsequent conjugation with acetic or 2-oxo-propionic acids. Separation of the thiazole and oxadiazine rings was only a minor pathway in ruminants and poultry.

In ruminants, the MARC concluded (meeting held on 7/28/99) that the residue of concern is parent + CGA-322704 metabolite. The parent thiamethoxam and the CGA-322704 metabolite accounted for a high percentage of the radioactivity identified in milk and muscle (the commodities with the highest dietary impact for humans) from the lactating goat study. The Committee was potentially concerned about the relatively high levels of metabolites MU-12 and N-5 in kidney and liver. However, after consulting with Alberto Protzel, the Committee concluded that these metabolites, which contain the chloro-thiazole ring but not the nitro group, would not need to be included in the tolerance expression or quantitatively used in a human health risk assessment.

In poultry, based on cursory review of the laying hen metabolism study and dietary burdens, a hen feeding study and establishment of poultry commodity tolerances will not be needed for the proposed uses. If additional uses are requested later which increase the dietary burden enough to require that a laying hen feeding study be conducted, the Committee concluded that the petitioner will need to analyze for the additional metabolite CGA-265307 (in addition to the parent thiamethoxam and CGA-322704 metabolites) based on it being the major residue in eggs and fat and containing the N-nitro group. For the reasons stated under the ruminants section for MU-12 and N-5, the MARC concluded that the metabolite MU-3 would not need to be included in the tolerance expression or quantitatively used in a human health risk assessment.

OPPTS GLN 860.1340: Residue Analytical Method

Proposed Tolerance Enforcement Method - animal and plant commodities

Novartis Crop Protection is proposing an HPLC/UV or MS method (Method AG-675) for enforcing tolerances for residues of thiamethoxam and its metabolite CGA-322704 in/on animal and crop commodities. A method description and method validation data were submitted in the following volume:

> 44703524 Campbell, D. (1998) Analytical Method for the Determination of Residues of CGA-293343 and the Metabolite CGA-322704 in Animal and Crop Substrates by High Performance Liquid Chromatography with Detection by UV and Mass Spectrometry, Including Validation Data: Lab Project Number: AG-675: 206-97: 346001.Unpublished study prepared by Novartis Crop Protection, Inc.235 p.

HPLC/UV Method AG-675: Residues of thiamethoxam and CGA-322704 in **fruits**, **vegetables**, and **grains** are extracted using ACN:water (80:20, v:v), filtered, and concentrated to an aqueous remainder. Residues in the aqueous phase are purified by reverse-phase (RP) SPE on a phenyl column eluted with MeOH:water (1:1, v:v), and concentrated. The residues are salinized, partitioned into EtOAc, concentrated to dryness, redissolved in EtOAc:hexane (20:80, v:v), and then further purified by sequential normal-phase (NP) SPE through amino and alumina columns eluted with MeOH:EtOAc (3:97 and 10:90, v:v, respectively). The eluate from the alumina column is evaporated to dryness, reconstituted in a mobile phase of hexane:EtOAc:PrOH:MeOH

(11:3:1:1, v/v), and analyzed by NP HPLC analysis using a Spherisorb S5 NH_2 column with UV detection at 255 nm.

Residues of thiamethoxam and CGA-322704 in **animal commodities** and **plant oils** are extracted using ACN:water (80:20, v:v), filtered (except oils), and partitioned with aqueous NaCl:hexane:toluene (5:2:20, v/v). The hexane layer is discarded, and residues in the aqueous layer are partitioned with ACN:toluene (98:2, v/v). The combined ACN/toluene extracts are concentrated to an aqueous remainder, and are then cleaned-up and analyzed as described above for fruits, vegetables, and grains.

HPLC/MS Method AG-675: Residues of thiamethoxam and CGA-322704 in **cotton, tobacco,** and **forage, fodder, and straw of cereal grains** are extracted using ACN:water (80:20, v:v), filtered, and concentrated to an aqueous remainder. Residues in the aqueous phase are adjusted to pH 7 using sodium phosphate buffer, and cleaned-up on a strong anion exchange SPE column. The residues are further purified on a phenyl SPE column eluted with MeOH:water (1:1,v:v), concentrated, salinized, partitioned into EtOAc, and concentrated to dryness. The residues are redissolved in EtOAc:hexane (20:80, v:v), purified on a NP alumina SPE column eluted with MeOH:EtOAc (10:90, v:v), and concentrated to dryness. The residues are then reconstituted in ACN:water (10:90, v:v) and analyzed by reverse-phase HPLC using a C_{18} column with a gradient of water to ACN with 0.1% acetic acid, and MS detection (m/z = 292 and 250 for thiamethoxam and CGA-322704, respectively).

The validated LOQ for residues of both thiamethoxam and its metabolite CGA-322704 is 0.01 ppm for all matrices with the exceptions of milk and fruit juices (0.005 ppm) and grass (0.05 ppm). Method recovery data are presented in Table 23. For method validation, control samples of representative plant and animal matrices were fortified with thiamethoxam and CGA-322704 at 0.005-2.0 ppm. Overall average recoveries of each analyte from each matrix were 70-113%.

The petitioner also provided method validation data for CGA-265307, a metabolite of thiamethoxam found in livestock commodities. Control milk samples were fortified with CGA-265307 at 0.005 and 0.5 ppm, and were analyzed using HPLC/UV method AG-675 without modification. Recoveries of CGA-265307 were 85%, 92%, and 133%. Representative chromatograms and sample calculations were provided.

Table 23. Method validation recoveries of thiamethoxam and its metabolite CGA-322704 from fortified samples of plant and animal commodities using HPLC/UV (or MS) Method AG-675.

Commodity	Analysis Method	Fortification (ppm)	Thiamethoxam		CGA-322704	
			% Recovery [a]	Ave.	% Recovery [a]	Ave.
Plant Commodities						
Apple, wet pomace	HPLC/UV	0.01-1.0	69.3; 72.2-76.9 (3)	73.0	73.4-80.7 (4)	77.9
Apple, juice	HPLC/UV	0.005-0.5	82.4-89.1 (5)	85.6	75.8-87.7 (5)	82.1
Broccoli	HPLC/UV	0.01-0.2	76.3-101.2 (4)	87.8	81.6-94.9 (4)	89.8
Corn, grain	HPLC/UV	0.01, 0.5	86.6, 93.3	90.0	107.6, 117.4	112.5
Corn, fodder	LC/MS	0.01, 1.0	72.0-85.3 (3)	77.4	64.0; 73.8, 80.9	72.9
Cotton, seed	LC/MS	0.01-2.0	57.8; 70.0-75.4 (4)	69.8	69.6; 75.3-89.0 (4)	77.2
Cotton, seed oil	HPLC/UV	0.01-0.5	61.8, 66.4; 89.6-100.1 (3)	82.3	60.2, 68.5; 95.8-107.6 (3)	85.6
Cucumbers	HPLC/UV	0.01-0.5	83.4-91.3 (3)	87.2	89.9, 92.3	91.1
Grass	LC/MS/MS	0.05-0.5	78.0-101.0 (10)	87.4	75.2-97.6 (10)	86.2
Pears	HPLC/UV	0.01-0.5	85.3-103.6 (4)	95.2	88.2-108.3 (4)	97.4
Pepper, green	HPLC/UV	0.01-1.0	84.5-94.7 (5)	89.2	86.4-92.4 (5)	89.9
Potato, tubers	HPLC/UV	0.01-0.5	83.4-96.1 (5)	88.7	87.8-99.7 (5)	93.0
Potato, wet peel	HPLC/UV	0.01-1.0	78.7-88.2 (5)	84.1	78.0-90.3 (5)	85.2
Sorghum, forage	LC/MS	0.01-1.0	68.0; 82.0-86.0 (4)	80.6	78.0-96.0 (5)	86.0
Spinach	HPLC/UV	0.01-0.5	79.3-117.2 (5)	97.9	42.2; 77.4-93.2 (4)	77.1
Tobacco, green	LC/MS	0.01-1.0	91.6-96.1 (5)	93.8	77.5-85.2 (5)	82.1
Tomato	HPLC/UV	0.01-1.0	68.2; 74.8-110.3 (4)	85.5	86.8-104.7 (5)	94.5
Tomato, paste	HPLC/UV	0.01-2.0	76.4-79.1 (5)	77.7	77.0-83.6 (5)	81.0
Wheat, grain	HPLC/UV	0.01-0.5	78.3-102.2 (5)	90.6	77.9-94.3	88.3
Animal Commodities						
Cow, fat, omental	HPLC/UV	0.01-2.0	79.1-86.3 (5)	82.8	85.2-90.0 (50)	86.8
Cow, kidney	HPLC/UV	0.01-1.0	82.8-91.4 (4)	862	87.0-94.4 (4)	89.6
Cow, liver	HPLC/UV	0.01-0.5	84.3-90.1 (5)	86.0	86.0-91.6 (5)	89.3
Goat, milk	HPLC/UV	0.005-0.5	87.8-112.6 (3)	101.5	89.7-95.9 (3)	93.8
Goat, muscle	HPLC/UV	0.01-1.0	86.0-88.1 (3)	86.7	88.1-89.1 (3)	88.5
Poultry, eggs	HPLC/UV	0.01-2.0	81.2-91.9 (4)	85.0	85.4-94.8 (4)	89.3
Poultry, fat	HPLC/UV	0.01-1.0	83.3-97.9 (5)	88.5	89.2-94.0 (5)	92.6

[a] Each recovery value represents one sample unless otherwise indicated in parentheses. Recovery values outside the acceptable 70-120% range are listed separately.

The petitioner also submitted confirmatory method validation data for Method AG-675. Final extracts generated in the method validation study for AG-675 (described above) and originally analyzed by HPLC/UV and HPLC/MS were reanalyzed by HPLC/MS and HPLC/MS/MS, respectively, as the confirmatory methods. In addition, samples of cucumber, corn fodder, and goat milk from the metabolism studies bearing aged residues of thiamethoxam and CGA-322704 were analyzed by both methods to generate comparative recovery data. The confirmatory method validation and comparative recovery data are presented in Tables 24 and 25. The results of the confirmatory methods were in good agreement with the results obtained by the primary HPLC/UV or MS analyses.

Table 24. Method validation recoveries of thiamethoxam and CGA-322704 from fortified samples of animal and plant matrices analyzed by the proposed HPLC/UV or MS enforcement method, AG-675, and by a confirmatory method.

Commodity	Fortification Level (ppm)	# of Samples	% Recovery [a]	
			Primary Method (HPLC/UV or MS) [b]	Confirmatory Method (HPLC/MS or MS/MS) [b]
Thiamethoxam				
Corn, fodder	0.01, 1.0	3	72-85	75-83
Cucumber	0.01, 0.5	3	83-91	84-116
Goat, milk	0.005, 0.50	3	88-113	92-122 (1)
Sorghum, forage	0.01-1.0	5	68-86 (1)	85-96
Tobacco, green	0.01-1.0	5	92-96	73-88
CGA-322704				
Corn, fodder	0.01, 1.0	3	64-81 (1)	56-80 (2)
Cucumber	0.01, 0.5	3	90-173 (1)	87-94
Goat, milk	0.005, 0.50	3	90-96	89-120
Sorghum, forage	0.01-1.0	5	78-96	81-93
Tobacco, green	0.01-1.0	5	77-85	75-90

[a] Value in parentheses represents the number of recoveries outside the acceptable range (70-120%).

[b] Cucumbers and goat milk were analyzed by HPLC/UV and confirmed by HPLC/MS; all other matrices were analyzed by HPLC/MS and confirmed by HPLC/MS/MS.

Table 25. Comparative recoveries of thiamethoxam and CGA-322704 from metabolism study samples using HPLC/UV or MS method AG-675 and a confirmatory method.

Commodity	Residues Found (ppm)		Comparative % Recovery [b]
	Primary Method (HPLC/UV or MS) [a]	Confirmatory Method (HPLC/MS or MS/MS) [a]	
Thiamethoxam			
Cucumber	0.043	0.044	98
	0.051	0.047	109
	0.044	0.044	100
	0.022	0.025	88
	0.036	0.037	97
	0.038	0.033	115
Goat milk	0.087	0.098	89
	0.064	0.077	83
	0.085	0.095	89
Corn, fodder	0.022	0.022	100
	0.024	0.024	100
	0.025	0.025	100
CGA-322704			
Goat milk	0.17	0.16	106
	0.12	0.13	92
	0.16	0.15	107
Corn, fodder [c]	0.017	0.012	142
	0.021	0.014	150
	0.023	0.014	164

[a] Corn fodder samples were analyzed by HPLC/MS and recoveries confirmed by HPLC/MS/MS.
[b] Comparative recovery = residues found by primary analysis ÷ residues found by confirmatory method.
[c] A meaningful comparative analysis is doubtful as residues of CGA-322704 are at/near the LOQ (0.01 ppm). Residues of CGA-322704 in cucumber samples (not shown) were at or below the LOQ.

Radiovalidation: The petitioner also submitted radiovalidation data (MRID 44703524) for the proposed enforcement method to demonstrate the efficiency of the method in extracting and recovering residues of thiamethoxam and CGA-322704 from aged samples.

Radiolabeled samples of pears, corn grain and fodder, cucumbers, and goat meat and milk from the respective metabolism studies were analyzed using the proposed enforcement method and the results were compared with data from the metabolism studies (Table 26).

For comparison, the petitioner summarized data from the metabolism studies on the extractability of radioactivity (%TRR) and the level (ppm) of thiamethoxam and CGA-322704 in metabolism study samples. Although these results were in general agreement with the results from metabolism studies discussed above, there were minor differences in the data. In addition, the

petitioner did not report the TRR levels in the individual subsamples used for analysis, except for goat meat and milk. Calculation of the TRR levels by HED indicates that the subsamples used of analysis by Method AG-675 had different levels of radioactivity than the subsamples analyzed in the metabolism studies. Therefore, the %TRR values were used to compare the results of Method AG-675 with the metabolism study data.

Table 26. Radiovalidaton recoveries of thiamethoxam and its metabolite CGA-322704 from radiolabeled samples from metabolism studies analyzed using the proposed HPLC/UV or MS enforcement method.

Results from Metabolism studies							
Sample	TRR [a] (ppm)	Extractability (% TRR)	Thiamethoxam		CGA-322704 [b]		Combined recovery (% TRR)
			%TRR	ppm	% TRR	ppm	
Pear fruit	0.705	83.2	28.4	0.20	19.9	0.14	48.3
Corn grain	0.076	38.5	7.9	0.006	9.2	0.007	17.1
Corn fodder	0.870	57.8	5.7	0.05	3.4	0.03	9.2
Cucumbers	0.352	99.5	39.8	0.14	2.8	0.01	42.6
Cucumbers	0.154	86.9	13.0	0.02	1.9	0.003	14.9
Goat meat	2.07	94.7	48.3	1.0	5.8	0.12	54.1
Goat milk	1.17	91.2	31.6	0.37	37.6	0.44	69.2
Results from Method AG-675 analyses [c]							
Sample	TRR [a] (ppm)	Extractability (% TRR)	Thiamethoxam		CGA-322704 [b]		Combined recovery (% TRR)
			%TRR	ppm [d]	% TRR	ppm [d]	
Pear fruit	0.663	82.4	25.2	0.167	13.1	0.087	38.3
Corn grain	-- [e]	33.9	--	<0.01	--	<0.01	--
Corn fodder	0.815	40.1	2.9	0.024	2.5	0.020	5.4
Cucumbers	0.328	57.0	14.0	0.046	--	<0.01	14.0
Cucumbers	0.303	73.5	10.6	0.032	4.6	0.014	15.2
Goat meat	1.68	87.9	42.3	0.71	3.1	0.052	45.4
Goat milk	0.394	86.4	20.1	0.079	38.1	0.15	58.2

[a] TRR values for samples were calculated by the reviewer.
[b] Residues of CGA-322704 are expressed in terms of thiamethoxam.
[c] All samples were analyzed using HPLC/UV, except corn fodder which was analyzed by HPLC/MS.
[d] Data are the average of triplicate analyses.
[e] -- indicates values which could not be determined as residues of thiamethoxam and/or CGA-322704 were <LOQ.

Solvent extraction using Method AG-675 released 34-88% of the TRR from the plant and animal samples. With the exceptions of corn fodder and one of the cucumber samples, extractability of

radioactivity by AG-675 was equivalent to 85-99% of the radioactivity extracted in the metabolism studies.

Due to the low level of residues (<0.01 pm) in corn grain a direct comparison of the two methods could not be made for this matrix. However, the results from the HPLC/UV analysis of pear, one of the cucumber samples, and the goat meat and milk samples are in good agreement with the results from the respective metabolism studies. The combined residues of thiamethoxam and CGA-322704 determined by Method AG-675 accounted for 15.2-58.2% of the TRR for these samples; based on the %TRR for each analyte, these values are equivalent to 79-102% of the combined residues determined in the metabolism studies. Although the recovery of residues from corn fodder using Method AG-675 was only 59% of the levels recovered in the metabolism study, the relative levels of thiamethoxam and CGA-322704 were low (5.4-9.2% TRR) for both analyses. For the one cucumber sample that had lower extractability and recovery of ^{14}C-residues, the petitioner attributed this to variability in the subsample rather than a difference between the methods.

The radiovalidation data for Method AG-675 are adequate for plant commodities; however, additional radiovalidation data are required to assess the efficiency of this method in recovering ^{14}C-residues from animal commodities. Although the available data indicate that thiamethoxam and CGA-322704 are adequately extracted and recovered from milk and meat using Method AG-675, data are required to assess the efficiency of this method in recovering thiamethoxam and CGA-322704 from radiolabeled liver samples. In the metabolism studies, metabolite CGA-322704 accounted for 6.4-7.2% TRR (0.7-0.8 ppm) in goat liver and 34-39% TRR% (2.7-3.5 ppm) in hen liver. In both studies, microwave extraction was required to release >90% of the CGA-322704 from liver.

Independent laboratory validation of Method AG-675: The petitioner submitted data (citation listed below) pertaining to independent laboratory validation of Method AG-675 used to determine residues of thiamethoxam and its metabolite CGA-322704 in/on animal and plant commodities.

> 44703522 Crawford, C. (1998) Independent Laboratory Validation of Method AG-675, for the Determination of Residues of CGA-293343 and the Metabolite CGA-322704 in Animal and Crop Substrates: Lab Project Number: 490-98: 7693-98-0174-CR-001: AG-675. Unpublished study prepared by Ricerca, Inc. 81 p.

The ILV trial was conducted by Ricerca, Inc. (Painsville, OH). Samples of untreated animal and plant commodities were fortified with thiamethoxam and its metabolite CGA-322704 each at the LOQ (0.01 ppm; 0.005 ppm for milk), and at the proposed tolerances or 10x the LOQ. Apple wet pomace, cotton, and wheat samples were control samples from field trials; eggs, milk, beef liver, lettuce, and tomato samples were obtained locally. Samples of eggs, milk, beef liver, apple wet pomace, cotton seed, lettuce, and tomatoes were quantitated by HPLC/UV, and samples of wheat grain, straw, and forage were quantitated by HPLC/MS. All test matrices were

successfully validated in the first trial, except for eggs where two trials were required before successful validation recoveries were obtained. The initial trial was unsuccessful due to excessive foaming during the initial rotary evaporation step thus not reducing the sample size adequately for the first cleanup column. Following consultation with the petitioner, minor modifications to the method were incorporated: reduced test aliquot, reduced solvent amounts used in the initial partitioning step, and increased the final volume for quantitation. The laboratory indicated that a set of seven samples (including reagent blank) required approximately 10 person hours to complete.

The results of the independent laboratory validation studies are presented in Table 27. Apparent residues of thiamethoxam and CGA-322704 were each <LOQ (<0.005 ppm or <0.01 ppm) in/on all control samples with the exception of two wheat straw samples that bore apparent residues of CGA-322704 at 0.004 and 0.005 ppm. Adequate recoveries were obtained at all fortification levels; therefore, no additional independent laboratory validation data will be required to support this petition.

Table 27. Independent laboratory validation of HPLC/UV and LC/MS methods using fortified untreated animal and plant samples.

Commodity	Fortification Level (ppm)	% Recovery	
		Thiamethoxam	CGA-322704
Apple, wet pomace	0.01	110, 110	110, 110
	0.2	87, 90	89, 91
Cotton, seed	0.01	100, 110	80, 80
	0.1	100, 103	93, 99
Eggs	0.01	100, 100	110, 120
	0.1	92, 95	97, 99
Lettuce	0.01	100, 110	80, 90
	2.0	92, 100	94, 101
Liver, beef	0.01	100, 110	100, 120
	0.1	87, 88	90, 92
Milk, whole	0.005	100, 100	100, 100
	0.02	100, 100	100, 100
Tomatoes	0.01	90, 90	100, 120
	0.1	82, 100	83, 102
Wheat, grain	0.01	100, 120	110, 120
	0.1	94, 94	96, 97
Wheat, forage	0.01	100, 100	90, 100
	0.1	82, 85	84, 89

Wheat, straw	0.01	90, 90	100, 100 [a]
	0.1	88, 88	90, 94 [a]

[a] Values were corrected for apparent residues (0.004 and 0.005 ppm) in the control sample.

Residue Data Collection Methods

The petitioner also submitted a method description and validation data (citation listed below) for an earlier version of the proposed enforcement method, HPLC/UV method REM 179.01 used for determining residues of thiamethoxam *per se* in/on plant commodities.

44703536 Mair, P. (1995) Determination of CGA-293343 By HPLC (REM 179.01): Lab Project Number: REM 179.01: 1012-98. Unpublished study prepared by Novartis Crop Protection AG. 24 p.

Method REM 179.01: Briefly, residues of thiamethoxam in crop matrices are extracted with water:MeOH (80:20, v:v), filtered, diluted with water, and sequentially purified on phenyl SPE and carbon cartridges. The residues are eluted from the carbon column with water:THF (8:2, v:v), concentrated to dryness, and redissolved in water. The residues are then analyzed by RP HPLC analysis using a two-column switching system (C_{18} to phenyl) with UV detection (255 nm) and isocratic mobile phases of water:MeOH (8:2 and 7:3, v:v, respectively). The validated LOQ for residues of thiamethoxam in/on crop matrices is 0.02 ppm with the exception of corn whole plant and stem, and sugar beet tops, for which the LOQ is 0.5 ppm.

The petitioner submitted method validation data on representative plant matrices (Table 28). For method validation, untreated samples of apple, cabbage, corn, pea, potato, sugar beet, and tomato commodities were fortified with thiamethoxam at 0.02-0.5 ppm, and analyzed using HPLC/UV Method REM 179.01. Overall method recoveries of thiamethoxam were 71-110%. Sample calculations and representative chromatograms were provided.

Table 28. Method validation recoveries of thiamethoxam from fortified samples of various crop matrices using HPLC/UV Method REM 179.01.

Commodity	Fortification Level (ppm)	# of Samples	% Recovery Thiamethoxam	Average % Recovery
Apple	0.02, 0.2	6	84-97	91
Cabbage, heads	0.02, 0.2	6	88-110	96
Corn, grain	0.02, 0.2	19	79-102	89
Corn, stem	0.05, 0.5	6	76-98	82
Corn, whole plant	0.05, 0.5	6	71-87	82
Pea, seed	0.02, 0.2	6	82-101	93

(continued; footnotes follow)

Commodity	Fortification Level (ppm)	# of Samples	% Recovery Thiamethoxam	Average % Recovery
Pea, empty pods	0.02, 0.2	6	82-91	86
Potato	0.02, 0.2	6	82-99	90
Sugar beet, root	0.02, 0.2	6	80-86	83
Sugar beet, tops	0.05, 0.5	6	90-107	98
Tomato	0.02, 0.2	6	82-93	87

Canola

Samples of canola seed from the principal U.S. field trials (1998; MRID 44703527) were analyzed for residues of thiamethoxam and CGA-322704 using a modification of the proposed enforcement method AG-675, described above. A different extraction mix (ACN:hexane) was used and two cleanup steps were eliminated because of the use of a more selective detection system (LC/MS/MS using Single Ion Monitoring). The validated LOQ for each analyte in/on canola seed is 0.01 ppm. Samples were analyzed by Enviro-Test Laboratories (ETL; Edmonton, Alberta, Canada).

Samples of canola and mustard seed from the additional field trials (1998; MRIDs 447035-28 to -30) were analyzed for residues of thiamethoxam and CGA-322704 using a turbo ion spray LC/MS/MS method developed by ETL. Briefly, residues in canola seed are extracted with ACN:hexane and centrifuged; residues remaining in the hexane layer are extracted with ACN. Residues in the combined ACN extracts are concentrated, diluted with water, and purified on a C_{18} SPE cartridge eluted with MeOH. The residues are then analyzed by HPLC/MS/MS using a C_{18} column with an isocratic mobile phase of 0.1M acetic acid:ACN (40:60, v/v), and MS detection. The validated LOQs for residues of each analyte in/on canola and mustard seed are 0.025 and 0.05 ppm, respectively. Samples were analyzed by ETL.

The petitioner submitted method validation data in conjunction with the field trial studies using samples of canola and mustard seed fortified at 0.01-0.5 ppm. Method recoveries are presented in Table 29. Adequate recoveries were obtained for each analyte from both matrices. Apparent residues of each analyte were <LOQ (<0.01-0.05 ppm) in/on all control samples of canola and mustard seed.

Table 29. Method recoveries of thiamethoxam and CGA-322704 from fortified samples of plant and animal commodities using HPLC/MS [a].

MRID	Commodity	Fortification (ppm)	Thiamethoxam		CGA-322704	
			% Recovery [b]	Ave.	% Recovery [b]	Ave.
Method Validation Recoveries						
44703528	Canola seed	0.05-0.5	62, 75-101 (5)	85	68, 76-99 (5)	84
44703530		0.025-0.5	64, 73-105 (8)	83	61, 67, 71-113 (7)	87
44703529	Mustard seed	0.05-0.5	114-120 (4), 123, 123	119	86-110 (6)	102
Concurrent Method Recoveries						
44703527	Canola seed	0.01-0.5	76-106 (6)	91	75-103 (6)	88
44703528		0.15, 0.5	47, 86	67	62, 85	74
44703530		0.025-0.15	66, 78-105 (8)	84	74-119 (4), 121	108
44703529	Mustard seed	0.05-0.5	84-118 (4)	98	79-98 (4)	92

[a] MRID 44703527 depicts concurrent recovery data for method AG-675. All other recovery data shown were generated using the turbo ion spray LC/MS/MS data collection method developed by ETL.

[b] Each recovery value represents one sample unless otherwise indicated in parentheses. Recovery values outside the acceptable 70-120% range are listed separately.

Conclusions: **Plants Commodities.** Novartis HPLC/UV (or MS) Method AG-675 (MRID# 447035-24) is adequate for collecting data on residues of thiamethoxam and CGA-322704 in/on canola and mustard seed. Adequate method validation data were submitted for canola seed (in MRID# 447035-27) and various additional crop matrices. Method AG-675 has been adequately radiovalidated, and has undergone a successful (independent laboratory validation (ILV) trial. The validated limit of quantitation (LOQ) for residues of each analyte is 0.01 ppm in all plant matrices with the exception of fruit juices (0.005 ppm) and grass (0.05 ppm).

Based on achieving adequate recoveries on canola using the LC/MS method (MRID# 447035-27), the petitioner has not adequately explained why the majority of the canola field trial data were analyzed by a LC/MS/MS method. It appears that there may have been some interference problems and, rather than modify or add clean-up steps to eliminate the interference, the petitioner chose to **eliminate 2 clean-up steps** and use a more selective detection system (LC/MS/MS using single ion monitoring). The LC/MS/MS is the proposed enforcement method for canola. RAB2 has reservations about proposing an enforcement method which uses equipment most enforcement laboratories do not yet have access to in lieu of better clean-up procedures. In addtion, no ILV was submitted for the LC/MS/MS method. However, RAB2 will defer to the Analytical Chemistry Branch (ACB) of BEAD for their recommendations concerning this issue. Their comments will be available when they complete the PMV.

A petition method validation (PMV) request has been submitted to the Analytical Chemistry Branch (ACB) of BEAD (see memo of G.J. Herndon dated 9/28/99). RAB2 requested that ACB use the proposed enforcement methods (MRID# 447035-24 and 447035-27) to validate recovery

of thiamethoxam and its metabolite CGA-0322704 from canola, cotton, tomato, spinach, wheat grain, milk, beef liver, and eggs. The results of the PMV request have not been received.

With the submission of a method having both UV and MS detection, the issues of method specificity and confirmatory procedures have been adequately addressed.

Animal Commodities. Adequate method validation data using animal commodities have been submitted for Novartis HPLC/MS Method AG-675, and the method has undergone a successful ILV trial using milk, eggs, and beef liver. The validated LOQ for residues of thiamethoxam and CGA-322704 is 0.01 ppm each in meat, poultry, and eggs, and 0.005 ppm each in milk. This method has also been adequately radiovalidated using samples of meat and milk from the goat metabolism study. However, additional radiovalidation data are required in order to assess the efficiency of this method in recovering thiamethoxam and CGA-322704 from beef liver. This will be required prior to the establishment of liver and/or meat-byproduct tolerances. In both the ruminant and poultry metabolism studies, microwave extraction was required to release CGA-322704 from liver.

A petition method validation (PMV) request has been submitted to the Analytical Chemistry Branch (ACB) of BEAD (see memo of G.J. Herndon dated 9/28/99). RAB2 requested that ACB use the proposed enforcement methods (MRID# 447035-24 and 447035-27) to validate recovery of thiamethoxam and its metabolite CGA-322704 from canola, cotton, tomato, spinach, wheat grain, milk, beef liver, and eggs. The results of the PMV request have not been received.

Although additional data are required to assess the suitability of Method AG-675 for determining residues in beef liver, this requirement does not impact this petition as animal feeding studies and animal tolerances are not required to support the proposed use on canola.

OPPTS GLN 860.1360: Multiresidue Methods

The petitioner submitted data (citation listed below) concerning the recovery of residues of thiamethoxam using FDA multiresidue method protocols (PAM Vol. I). These data will be forwarded to FDA for evaluation.

> 44703523 Lin, K. (1998) Determination of CGA-293343 by U.S. Food and Drug Administration Mutiresidue (sic) Method Testing: Lab Project Number: ABR-98054: 363-96: 346001. Unpublished study prepared by Novartis Crop Protection, Inc. 50 p.

Recovery of thiamethoxam was 50-60% using Protocol D and <30% using Protocol E. Using Protocol C, thiamethoxam obtained adequate detector responses to Section 302 DG5 and DG13 gas-liquid chromatography (GLC) systems. Metabolites CGA-322704 and CGA-265307 were tested using Protocol C, but did not yield adequate detector responses to any of the Section 302 DG5, DG13, and DG18 GLC systems; no further testing was conducted for the metabolites.

In the memos of G.J. Herndon dated 9/28/99, the results of the multiresidue testing were forwarded to FDA (Mark Wirtz) and ACB/BEAD (Francis Griffith).

OPPTS GLN 860.1380: Storage Stability Data - Plants

Novartis submitted the following studies investigating the frozen storage stability of residues of thiamethoxam and its metabolite CGA-322704 in/on various plant RAC and processed commodities.

> 44703525 Mair, P. (1998) Stability of Residues of CGA-293343 (2-year Final Report) and CGA-322704 (1-year Interim Report) in Plant Material Under Deep Freezer Conditions, Including Method Validation: Lab Project Number: 112/96: 504-96: 103/98. Unpublished study prepared by Novartis Crop Protection AG. 65 p.
>
> 44715113 Grunenwald, M. (1998) Stability of CGA-293343 and CGA-322704 in Crops and Processed Fractions Under Freezer Storage Conditions: Lab Project Number: ABR-98103: 269-98: 346001. Unpublished study prepared by Novartis Crop Protection, Inc. 104 p.

MRID 44703525: In this study, the petitioner provided data depicting the storage stability of residues of thiamethoxam and CGA-322704 in various crops stored frozen for up to two years. Control samples of tomato, potato, rape seed, and corn grain were fortified with thiamethoxam or its metabolite CGA-322704 at ~0.1-0.6 ppm; actual fortification levels of each analyte were determined by the 0-day analyses. Apple samples bearing field-incurred residues were also analyzed. The fortified samples, samples bearing weathered residues, and unfortified control samples were stored frozen at ≤-18 C. At various intervals (0-761 days for thiamethoxam; 0-378 days for CGA-322704), one control, two samples freshly fortified with thiamethoxam or CGA-322704 at 0.04-0.5 ppm, and three stored-fortified samples were analyzed for residues of thiamethoxam and CGA-322704 using HPLC/UV methods REM 179.01 and -03. A description of the Method REM 179.03 was not provided. Apparent residues of thiamethoxam and CGA-322704 in all control samples were <LOQ (<0.04 ppm or <0.05 ppm, respectively). Samples analyses were conducted by Novartis Crop Protection AG, Basel, Switzerland.

The results of the storage stability studies are presented in Table 30. The submitted data indicate that residues of CGA-322704 and thiamethoxam are stable stored at ≤- 18 C in apples, corn grain, potato, canola seed, and tomato for up to 1 and 2 years, respectively.

MRID 44715113: The petitioner also presented interim data from an on-going study demonstrating the stability thiamethoxam and CGA-322704 in plant RAC and processed commodities. Samples of untreated leaf lettuce, safflower seed, and processed canola oil, corn meal, and tomato puree were fortified with thiamethoxam and CGA-322704 each at 0.5 ppm, and stored frozen with unfortified control samples at -20 C. At intervals of 0, 2, and 4 months, one control and two freshly fortified and stored fortified samples were analyzed for residues of thiamethoxam and CGA-322704. Samples were analyzed by NHSD using a draft of the previously discussed HPLC/UV method (AG-675); methodology was the same as the final

version. Residue results were corrected for apparent residues in control samples. Apparent residues of each analyte were <0.05 ppm (<LOQ) in all control samples with the exception of one safflower seed sample which bore apparent CGA-322704 residues at 0.078 ppm.

The results of the interim storage stability study are presented in Table 30. The submitted data are adequate and indicate that residues of thiamethoxam and CGA-322704 are stable in canola oil, corn meal, leaf lettuce, safflower seed, and tomato puree for up to 4 months at -20 C.

Table 30. Stability of thiamethoxam and CGA-322704 in various plant matrices stored at ≤-18 C for up to 2 years.

MRID	Commodity	Storage Interval (months)	Fresh Fortification % Recovery	% Recovery Stored Samples [a]
Thiamethoxam				
44703525	Apple	0	87, 94	--
		1	86, 88	100, 100, 100
		4	89, 90	92, 100, 108
		6	65, 77	75, 75, 83
		12	82, 85	92, 92, 100
		24	90, 94	100, 108, 108
	Corn, grain	0	74, 87	--
		1	67, 76	74, 75, 81
		3	84, 86	82, 85, 88
		6	71, 77	69, 75, 84
		12	83, 87	81, 85
		24	83, 90	84, 87, 88
	Potato	0	86, 95	--
		1	81, 81	85, 91, 96
		3	85, 95	76, 85, 93
		6	79, 82	78, 82, 85
		12	87, 92	63, 84, 88
		24	93, 103	87, 88, 106
	Canola, seed	0	91, 104	--
		1	77, 80	63, 63, 63
		3	67, 89	67, 67, 70
		6	65, 70	70, 85, 85
		12	68, 84	74, 78, 78
		24	84, 91	100, 100, 104
	Tomato	0	94, 95	--
		1	86, 87	88, 103, 113
		3	74, 78	70, 80, 88
		6	76, 78	63, 66, 75
		12	92, 98	88, 102, 108
		25	105, 107	98, 105, 113

(continued; footnote follows)

Table 30. *Continued.*

MRID	Commodity	Storage Interval (months)	Fresh Fortification % Recovery	% Recovery Stored Samples [a]
44715113	Canola oil	0	91, 97	91, 96, 100
		2	95, 96	97, 98
		4	95, 96	97, 99
	Corn, meal	0	66, 75	74, 75, 78
		2	80, 86	83, 87
		4	64, 89	91, 91
	Lettuce, leaf	0	91, 100	99, 100, 101
		2	93, 97	95, 100
		4	99	104, 104
	Safflower, seed	0	112, 118	89, 99, 113
		2	90, 93	90, 96
		4	82	72, 88
	Tomato, puree	0	81, 85	93, 95, 105
		2	95, 100	84, 89
		4	102, 108	100, 100
CGA-322704				
44703525	Apple	0	73, 84	--
		1	82, 86	107, 107, 107
		3	79, 79	110, 110, 114
		6	75, 80	100, 103, 110
		12	81, 81	114, 114, 117
	Corn, grain	0	76	--
		1	60, 60	81, 84, 84
		3	70, 71	94, 100, 103
		6	75, 80	103, 109, 116
		12	77, 91	119, 119, 122
	Potato	0	72, 76	--
		1	68, 70	86, 92, 92
		3	71, 72	94, 100, 103
		6	79, 81	97, 103, 106
		12	84, 88	97, 106, 108
	Canola, seed	0	96, 105	--
		1	90, 108	128, 141, 144
		3	77, 91	97, 103, 109
		6	69, 73	72, 75, 78

(continued; footnote follows)

MRID	Commodity	Storage Interval (months)	Fresh Fortification % Recovery	% Recovery Stored Samples [a]
	Tomato	12	94, 106	97, 100, 106
44703525		0	70, 83	--
		1	88, 91	127, 130, 152
		3	74, 76	109, 130, 130
		6	73, 82	145, 155, 158
		12	73, 85	103, 109, 115
44715113	Canola oil	0	90, 104	94, 100, 106
		2	98, 99	49, 100
		4	98, 98	97, 98
	Corn, meal	0	69, 78	81, 83, 83
		2	84, 91	90, 92
		4	66, 94	92, 92
	Lettuce, leaf	0	94, 110	103, 104, 106
		2	92, 95	66, 102
		4	101	102, 102
	Safflower, seed	0	94, 96	81, 99, 109
		2	87, 88	86, 93
		4	84	85, 91
	Tomato, puree	0	89, 90	92, 99, 105
		2	97, 103	91, 95
		4	102, 108	103, 104

[a] Percent recoveries were calculated by the reviewer.

Conclusions: The submitted two-year storage stability study on thiamethoxam *per se* and one-year interim study on CGA-322704 are adequate pending submission of a detailed description of Method REM 179.03, used to determine residues of each analyte in some study samples. The available data indicate that residues of CGA-322704 and thiamethoxam are stable stored at ≤- 18 C in apples, corn grain, potato, canola seed, and tomato for up to 1 and 2 years, respectively. HED assumes that Method REM 179.03 is similar to Method REM 179.01 (described above), however, Method REM 179.03 is capable of determining residues of parent and CGA-322704.

Interim data from the on-going storage stability study are adequate, and indicate that residues of thiamethoxam and CGA-322704 are stable in/on canola oil, corn meal, leaf lettuce, safflower seed, and tomato puree for up to 4 months at -20 C.

Samples of canola and mustard seed from the residue field trials were stored frozen for 2-11 months from collection to analysis. The storage intervals and conditions of the residue studies are adequately supported by the storage intervals depicted in the available storage stability studies.

OPPTS GLN.860.1500: Crop Field Trials

Canola

Novartis Crop Protection submitted three studies depicting the concentration of residues of thiamethoxam and its metabolite CGA-322704 in/on canola seed grown from treated seed; data were also provided on residues of difenoconazole, fludioxonil, and mefenoxam (determined as 2,6-dimethylaniline; 2,6-DMA). The citations are listed below.

> 44703527 Vincent, T. (1998) CGA-293343 COMBI FS-D--Magnitude of the Residues in or on Canola: Lab Project Number: 476-97: OW-SR-301-97: 0S-SR-858-97. Unpublished study prepared by Novartis Crop Protection, Inc. 257 p.
>
> 44703528 Purdy, J. (1998) 8 Crop Residue Trials to Determine the Residues of CGA-169374, CGA-173506, CGA-293343, and CGA-329351 and Their Significant Crop Metabolites After Application of Helix as a Seed Treatment on Canola: Lab Project Number: 998-98: CER 03203/97: 98NOV32.REP. Unpublished study prepared by Novartis Crop Protection Canada Inc. 141 p.
>
> 44703530 Purdy, J. (1998) 10 Crop Residue Trials to Determine the Residues of CGA-169374, CGA-173506, CGA-293343, and CGA-329351 and Their Significant Crop Metabolites After Application of Helix as a Seed Treatment on Canola, 1998 Trials: Lab Project Number: 1009-98: CER03208/98: 98NOV35.REP. Unpublished study prepared by Novartis Crop Protection Canada Inc. 149 p.

In the first study (MRID 44703527), a total of eight canola tests (one test per location) were conducted between the 1997 and 1998 growing seasons in GA, ID, MN, MT, ND, OR, SD, and WA. Canola seed were treated using an RTU formulation (CGA-293343 Combi FS-D) at a nominal concentration of 500 g ai/100 kg of seed for thiamethoxam, 30 g ai/100 kg of seed for difenoconazole, 10 g ai/100 kg of seed for mefenoxam, and 3 g ai/100 kg of seed for fludioxonil (~1x the proposed maximum application rate). At the MN and ND test sites, two additional tests were conducted in which canola seed were treated with the test substance at a concentration of 1500 g ai/100 kg of seed for thiamethoxam, 90 g ai/100 kg of seed for difenoconazole, 30 g ai/100 kg of seed for mefenoxam, and 9 g ai/100 kg of seed for fludioxonil (~3x the proposed rate).

Samples of mature canola seed were harvested 87-295 days after planting. All samples, except samples for processing, were promptly frozen (temperature unspecified) and shipped by freezer truck to Novartis (Greensboro, NC). Canola samples for processing were shipped at ambient temperatures by overnight air freight to Engineering Bioscience Research Center of Texas A&M (Bryan, TX). After processing, samples were frozen and shipped by freezer truck to Novartis. The report stated that some samples were processed prior to storage, although no information as to processing procedures was reported. Samples (RAC and processed) were subsequently shipped on dry ice and delivered to Enviro-Test Laboratories (ETL; Edmonton, Alberta, Canada), where they remained under frozen storage conditions (-20±5 C) until residue analysis. The maximum storage intervals of samples from the canola field trials were 51-146 days (~2-5 months).

The petitioner elected to analyze samples from a reduced number of trials as no quantifiable residues were expected. Samples from the MT trial, which failed due to low yield, and from the OR trial were not analyzed.

Samples of canola seed were analyzed for residues of thiamethoxam and its metabolite CGA-322704 using a modification of the proposed enforcement method AG-675 (previously described). A different extraction mix (ACN:hexane) was used and two cleanup steps were eliminated because of the use of a more selective detection system (LC/MS/MS). Adequate concurrent recoveries of each analyte were obtained. Residues of thiamethoxam and CGA-322704 were each <0.01 ppm (<LOQ) in/on six control samples of canola seed, and in/on all samples of canola seed grown from seed treated at the ~1x rate (n=12) and ~3x rate (n=2).

In the second study (MRID 44703528), a total of eight canola tests were conducted during the 1997 growing season in the Canadian provinces of Alberta (4 tests), Saskatchewan (2 tests), and Manitoba (2 tests). Canola seed were treated using an RTU formulation (Helix™ FS-D) as a slurry at a nominal concentration of 500 g ai/100 kg of seed for thiamethoxam, 30 g ai/100 kg of seed for difenoconazole , 7 g ai/100 kg of seed for mefenoxam, and 5 g ai/100 kg of seed for fludioxonil (~1x the proposed rate). The control seed were treated with Vitavax RS® which contains carbathiin, thiram, and lindane.

Samples of mature canola seed were harvested 84-124 days after planting. All samples were promptly frozen (temperature unspecified) and shipped to ETL where they remained under frozen storage conditions (-10 C) until residue analysis, 286-347 days after collection.

Samples of canola seed were analyzed for residues of thiamethoxam and CGA-322704, difenoconazole, and fludioxonil using the adequate Turbo Ion spray LC/MS/MS method described above. Acceptable recoveries were obtained for thiamethoxam and CGA-322704. Recoveries from canola seed were 89 and 96% (n=2) for difenoconazole and 100 and 102% (n=2) for fludioxonil. Residues of thiamethoxam, CGA-322704, difenoconazole, and fludioxonil were each <0.05 ppm (<LOQ) in/on eight control samples of canola seed and 16 samples of canola seed grown from treated seed (~1x rate).

In the third study (MRID 44703530), a total of ten canola tests were conducted during the 1998 growing season. Eight trials were conducted in the Canadian provinces of Alberta (4 tests), Saskatchewan (2 tests), and Manitoba (2 tests), and two trials were conducted the Region 5 of the U.S. in MN and ND. Canola seed were treated using an RTU formulation (Helix™ 289 FS) as a slurry at a rate of 400 g ai/100 kg of seed for thiamethoxam, 24 g ai/100 kg of seed for difenoconazole, 7.8 g ai/100 kg of seed for mefenoxam, and 2.7 g ai/100 kg of seed for fludionoxil (1x the proposed rate). The application rates for two trials conducted at an exaggerated rates (~3x) were 1200 g ai/100 kg of seed for thiamethoxam, 72 g ai/100 kg of seed for difenoconazole, 23.4 g ai/100 kg of seed for mefenoxam, and 8.1 g ai/100 kg of seed of fludionoxil.

The RTU formulation (Helix™ FS-D) was modified between 1997 and 1998, and the new formulation used in this study, Helix™ 289 FS, contains a higher concentration of active ingredient and is thicker to promote drying on the seed. Side-by-side field trials comparing both formulations were conducted at three trial sites to ensure that the data from the 1997 trials are applicable to the overall registration. The control seed from the Canadian trial sites were treated with Vitavax RS®, which contains carbathiin, thiram, and lindane, and control seed from the U.S. trial sites were untreated.

Samples of mature canola seed were harvested 90-138 days after planting. All samples were promptly frozen (temperature unspecified) and shipped to ETL where they remained under frozen storage conditions (-20±5 C) until residue analysis, ~2.5 months after harvest.

Samples of canola seed were analyzed for residues of thiamethoxam and CGA-322704, difenoconazole, and fludioxonil using the adequate Turbo Ion spray LC/MS/MS method described above. Acceptable recoveries were obtained for thiamethoxam and CGA-322704. From canola seed samples fortified at 0.025 and 0.15 ppm, recoveries ranged from 76-84% (n=5) for difenoconazole and 79-101% (n=5) for fludioxonil . Residues of thiamethoxam, CGA-322704, difenoconazole, and fludioxonil were each <0.025 ppm (<LOQ) in/on 10 control samples of canola seed, 30 samples of canola seed grown from seed treated at ~1x with Helix™ FS-D (n=14) or 289 FS formulations (n=16), and eight samples of canola seed grown from seed treated with Helix™ FS-D at ~3x the proposed rate.

Geographic representation, reflecting the proposed use pattern, is adequate. According to OPPTS GLN 860.1500, a total of 8 trials are required for proposed uses on canola; however, data from only six trials is needed for the current petition as the proposed use of thiamethoxam resulted in no quantifiable residues. The petitioner conducted eight field trials on canola seed in Region 2 (GA; 1 trial), Region 5 (MN, ND; 4 trials), Region 7 (SD; 1 trial), and Region 11 (ID, WA; 2 trials). Of these trials, six support the proposed tolerance of 0.02 ppm; in two trials conducted in MN and ND, the method LOQ for residues of thiamethoxam and CGA-322704 at 0.05 ppm exceeds the proposed tolerance of 0.02 ppm. A total of 16 trials were conducted in

Canadian Regions 7 (2 trials), 7A (1 trial), and 14 (13 trials). Samples from the Canadian trials were also analyzed using methods with LOQs for residues of each analyte at 0.025 and 0.05 ppm.

Conclusions: The submitted canola field trial data from six tests conducted in the U.S. are adequate. Combined residues of thiamethoxam and CGA-322704 were below the combined LOQ (<0.02 ppm) in/on 12 samples of canola seed grown from seed treated with thiamethoxam at 500 g ai/100 kg seed (0.5 lb ai/100 lb seed; ~1x the maximum proposed use rate) and harvested at maturity, 87-295 days after planting. These data support the proposed tolerance of 0.02 ppm for residues of thiamethoxam and CGA-322704 in/on canola seed

The petitioner submitted residue data from additional canola field trials performed in Canada (16 tests) and the U.S. (2 tests). As samples from these tests were analyzed using methods for which the combined LOQ for residues of thiamethoxam and CGA-322704 (0.05 and 0.1 ppm) exceeds the proposed tolerance, the resulting residue data were not used to determine the adequacy of the proposed tolerance. Combined residues of thiamethoxam and CGA-322704 were below the combined LOQ (<0.05 or <0.1 ppm) in/on 46 samples of canola seed grown from seed treated with thiamethoxam at 400-500 g ai/100 kg seed (0.4-0.5 lb ai/100 lb seed; ~1x) and harvested at maturity, 84-138 days after planting.

Mustard

Novartis Crop Protection submitted a study depicting residues levels of thiamethoxam and its metabolite CGA-322704 in/on mustard seed grown from seed treated with the thiamethoxam MAI formulation, Helix™ 289-FS. Given the similarity of this use with the canola seed treatment, these data are reviewed in this petition although no tolerance or uses are currently being proposed on mustard grown for seed. As with the canola studies, the petitioner has also provided residue data on difenoconazole, fludioxonil, and of mefenoxam (detected as 2,6-DMA). The results are included in the following volume of data:

> 44703529 Purdy, J. (1998) 5 Crop Residue Trials to Determine the Residues of CGA-169374, CGA-173506, CGA-293343, and CGA-329351 and Their Significant Crop Metabolites After Application of Helix as a Seed Treatment on Mustard: Lab Project Number: 1008-98: CER03210/98: 98NOV36.REP. Unpublished study prepared by Novartis Crop Protection Canada Inc. 111 p.

A total of five field trials were conducted during the 1997 growing season in the Canadian provinces of Alberta (2 trials), Saskatchewan (2 trials) and Manitoba corresponding to Canadian Regions 7, 7A, and 14(3 trials). Mustard seed were treated using an RTU formulation (Helix™ 289-FS) at a nominal concentration of 400 g ai/100 kg of seed for thiamethoxam, 24 g ai/100 kg of seed for difenoconazole, 7.8 g ai/100 kg of seed for mefenoxam, and 2.7 g ai/100 kg of seed for fludioxonil. The control seed were treated with Vitavax R® which contains carbathiin, thiram, and lindane.

Samples of mature mustard seed were harvested 101-104 days after planting. All samples were promptly frozen (temperature unspecified) and shipped to ETL, where they remained under frozen storage conditions (-10 C) until residue analysis. Samples were analyzed with 2 months of collection.

Samples of mustard seed were analyzed for residues of thiamethoxam, CGA-322704, difenoconazole, and fludioxonil using the Turbo Ion spray LC/MS/MS method described above. Adequate recoveries were obtained for thiamethoxam and CGA-322704. Recoveries from mustard seed control samples fortified at 0.05-0.5 ppm were 100-112% (n=4) for difenoconazole and 67-120% (n=4) for fludioxonil. Residues of thiamethoxam, CGA-322704, difenoconazole, and fludioxonil were <0.05 ppm (<LOQ) in/on five control samples of mustard seed and 10 samples of mustard seed grown from treated seed (1x rate).

Samples of mustard seed were analyzed for residues of mefenoxam using Novartis method AG-395, described above, and concurrent recoveries from control mustard seed samples fortified at 0.05-0.5 ppm were 82-122% (n=4). Residues of mefenoxam, detected as 2,6-DMA, were <0.05 ppm (<LOQ) in/on five control samples of mustard seed, and 10 samples of mustard seed grown from treated seed (1x).

Comments: The combined residues of thiamethoxam and CGA-322704 were below the combined LOQ (<0.1 ppm) in/on 10 mustard seed samples grown from seed treated with thiamethoxam at 400 g ai/100 kg seed (0.4 lb ai/100 lb seed) and harvested at maturity, 101-104 days after planting.

OPPTS GLN 860.1520: Processed Food/Feed

In conjunction with the residue field trials on canola described above, a total of four tests were conducted using a RTU formulation of thiamethoxam (Helix™ 289-FS or FS-D) at ~3x the proposed rate. In two tests conducted in MN and ND (1998; MRIDs 44703527), residues of thiamethoxam and CGA-322704 were each <0.01 ppm (<LOQ) in/on two treated canola seed samples. In two additional tests conducted in MN and ND (1998; MRIDs 44703530), residues of thiamethoxam and CGA-322704 were each <0.025 ppm (<LOQ) in/on eight treated canola seed samples; as samples from these tests were analyzed using a method for which the LOQ for residues of thiamethoxam and CGA-322704 (0.025 ppm each) exceeds the proposed tolerance for residues in/on canola (0.02 ppm), these residue data were not used to determine the need for a processing data and tolerances in processed canola commodities. Residues of difenoconazole, mefenoxam, and fludioxonil in/on all samples analyzed (n=10) were each below the respective LOQs for each analyte (<0.01 or <0.025 ppm). Residue data on processed commodities was not provided.

Conclusions: As treatment at ~3x the maximum proposed application rate did not result in quantifiable residues of thiamethoxam and CGA-322704 in canola seed samples, and as residues

in/on canola seed from all field trials conducted at ~1x were <LOQ, no further processing data or tolerances for residues in processed commodities are required for canola. The maximum theoretical concentration factor for canola is 3x.

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

Ruminant and poultry feeding studies are not required for purposes of establishing a tolerance for thiamethoxam residues in/on canola seed. Considering only the proposed use on canola, the maximum theoretical dietary exposure for livestock would be 0.003 ppm for both livestock and poultry based on the proposed 0.02 ppm tolerance for the combined residues of thiamethoxam and CGA-322704 in/on canola seed. Accordingly, the ~100 ppm dose level used in the ruminant and poultry metabolism studies discussed above would reflect a >30,000x dose level. Based on the level of residues found in animal commodities in the metabolism studies at the 100 ppm dosing level, there is no reasonable expectation of finite residues being transferred to animal commodities from the proposed use of thiamethoxam on canola; therefore, tolerances for residues in animal commodities are not required at this time.

However, the petitioner has submitted a ruminant feeding study (1998, MRID 44703534) in support of establishing tolerances for thiamethoxam on a wide variety of commodities (PP#9F5046). The ruminant feeding study is currently being reviewed in conjunction with this other petition.

OPPTS GLN 860.1850: Confined Accumulation in Rotational Crops

Confined Rotational Crop Study I. With the current petition, Novartis has submitted two sets of studies on the accumulation of residues from [^{14}C]thiamethoxam in rotational crops. The first set of studies depicts the accumulation of ^{14}C-residues in confined rotational crops following an application of either [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at ~100 g ai/ha (0.09 lb ai/A) to the soil. The in-life phase of this study was conducted at Novartis' Midwest Research Station (MRS) in Dewey, IL; determinations of TRR in crop and soil samples were conducted at Novartis' Vero Beach Research Center (VBRC), FL, and the analytical analyses were conducted by Novartis' Human Safety Department (NHSD), Greensboro, NC. Results from this study are reported in:

> 44703531 Ray, W. (1998) Study on Rotational Crops After Soil Application of Thiazol-2-^{14}C-CGA-293343 and Oxadiazin-4-^{14}C-CGA-293343: Lab Project Number: ABR-98040: 82-96: 346001. Unpublished study prepared by Novartis Crop Protection, Inc. 128 p.

The [thiazol-2-^{14}C]thiamethoxam had a specific activity of 41.9 µCi/mg (93,018 dpm/µg) and a radiochemical purity of 98.7%, and the [oxadiazin-4-^{14}C]thiamethoxam had a specific activity of 48.3 µCi/mg (107,226 dpm/µg) and a radiochemical purity of 98.6%. Each test substance was dissolved in MeOH and applied to separate plots of a silty loam soil (13% sand, 51% silt, 36%

clay, and 3.6% organic matter; pH 7.3; and CEC of 15.8 meq/100 g). To simulate a seed treatment, the top 1-inch layer of soil was removed from each plot prior to treatment, and was returned to the appropriate plot following treatment. Each test substance was applied at a target rate of 100 g ai/ha (0.09 lb ai/A); actual application rates were 97 and 109 g ai/ha for the [thiazol-^{14}C] and [oxadiazin-^{14}C] test substances, respectively, equivalent to 0.09 and 0.10 lb ai/A. Control and treated plots remained fallow until rotational crops were planted.

The petitioner has indicated that the maximum proposed seasonal rate is equivalent to 0.17 lb ai/A reflecting a proposed use on fruiting vegetables; however, proposed uses of a 2 lb/gal FlC and a 25% WP formulation on cotton have the potential to result in a maximum seasonal rate of 0.215 lb ai/A (242 g ai/ha). Based on the potential seasonal maximum for cotton, this study was conducted at ~0.4x the maximum seasonal rate.

The control and treated plots were each subdivided into three subplots, one for each PBI. At 30, 120, and 365 days after treatment a subplot was planted with turnips, mustard, and wheat. For the 365-day PBI, spinach was planted instead of mustard. The crops received water, fertilizer, and maintenance pesticides as necessary; adequate information pertaining to the growing conditions was provided.

A single sample of each commodity was collected at each PBI from the control and treated plots. Wheat forage was harvested at 25% and 50% crop maturity, 28-55 and 44-261 days after planting (DAP), respectively. Mustard/spinach leaves were harvested 45-51 DAP, and turnips were harvested 51-76 DAP and separated into roots and leaves (tops). Wheat was also harvested at maturity, 79-309 DAP, and separated into grain and straw. In addition, soil samples (0-3") were collected before and after each application and at each planting interval. After collection, plant and soil samples were stored at ≤-15 C and held at MRS for 1-38 days prior to shipment by freezer truck to VBRC.

Total radioactive residues (TRR)

TRR determinations were conducted by VBRC within 1-2 month of sampling. Crop and soil samples were ground with dry ice and radioassayed in triplicate by LSC following combustion. Example calculations for determining the LOQ for the radioassays were provided, but the actual LOQ for each matrix was not stated. The TRRs in/on treated plant commodities are presented in Table 31. Radioactivity in control matrices was <0.001 ppm. Samples were stored frozen for a total of 8-36 days at VBRC and were then shipped to the analytical laboratory (NHSD) by freezer truck or by overnight courier on dry ice.

Radioactive residues were generally low (≤0.05 ppm) in plant samples, with the exception of wheat straw. The level of ^{14}C-residues resulting from application of the two ^{14}C-labels was also similar for each crop matrix. ^{14}C-Residues were lowest in turnip roots (<0.01 ppm) followed by wheat grain (0.006-0.019 ppm) and mustard/spinach leaves (0.012-0.023 ppm). Radioactive residues were 0.008-0.036 ppm in wheat forage and 0.021-0.169 ppm in straw. For each

commodity, ^{14}C-residues were generally highest at the 30-day PBI and lowest at the 120-day PBI. ^{14}C-Residues increased slightly in most commodities between the 120- and 365-day PBIs.

On the day of application, ^{14}C-residues were 0.085 and 0.105 ppm in the top 3 inches of soil from the [thiazol-^{14}C] and [oxadiazin-^{14}C]treated plots, respectively. At the 30-, 120-, and 365-day PBIs, ^{14}C-residues in soil were 0.070, 0.049, and 0.060 ppm from the [thiazol-^{14}C]treated plots and 0.056, 0.041, and 0.042 ppm from the [oxadiazin-^{14}C]treated plots.

Table 31. Total radioactive residues found in/on representative rotational crops grown in a silty loam soil treated with [^{14}C]thiamethoxam at ~100 g ai/ha (~0.4x the maximum proposed seasonal rate).

Crop/commodity	Plant-back Interval (days)	Sampling interval [a]		Total radioactive residues (ppm) [b]	
		DAT	DAP	[Thiazol-^{14}C]	[Oxadiazin-^{14}C]
Mustard Leaves	30	81	51	0.019	0.016
	120	165	45	0.015	0.023
Spinach Leaves	365	413	48	0.017	0.012
Turnip Tops	30	81	51	0.051	0.045
	120	172	52	0.014	0.016
	365	441	76	0.026	0.027
Turnip Roots	30	81	51	0.008	0.006
	120	172	52	0.004	0.003
	365	441	76	0.003	0.003
Wheat forage (25% mature)	30	58	28	0.036	0.022
	120	175	55	0.018	0.024
	365	413	48	0.015	0.015
Wheat forage (50% mature)	30	74	44	0.036	0.031
	120	381	261	0.008	0.010
	365	421	56	0.021	0.029
Wheat straw	30	109	79	0.169	0.113
	120	429	309	0.026	0.021
	365	452	87	0.050	0.045
Wheat grain	30	109	79	0.019	0.017
	120	429	309	0.006	0.007
	365	452	87	0.009	0.010

[a] Crop sampling intervals are expressed in terms of days after soil treatment (DAT) and day after planting.
[b] Data are expressed in [^{14}C]thiamethoxam equivalents and are the average of triplicate analyses.

Storage stability data

After collection, plant samples for this confined rotational crop study were stored frozen for 2.2-7.8 months prior to the initial extraction and characterization of ^{14}C-residues, with most samples being extracted and analyzed within 4-6 months of harvest.

To support the stability of ^{14}C-residues during storage, the petitioner reextracted and analyzed [thiazol-^{14}C]-treated samples of wheat straw and mustard leaves and [oxadiazin-^{14}C]-treated samples of wheat grain and straw and turnip leaves from the 30-day PBI. These samples, which were originally extracted for analysis after 4.9-7.3 months of frozen storage, were reextracted after an additional 11-14 months of storage at the end of the study. Extractable ^{14}C-residues from the two extractions were analyzed by 2D-TLC and compared.

Although there were some differences in the chromatographic profile from the two time points for each matrix, the overall metabolite profile was similar over time. No additional storage stability data are required to support this confined rotational crop study.

Extraction of residues

Extraction and characterization of ^{14}C-residues were conducted at NHSD. Prior to analysis, plant samples were stored at -23 C at NHSD. Plant samples were stored frozen for a total of 70-238 days (2.2-7.8 months) prior to the initial extraction for analysis.

Plant samples with TRR values of ≤0.01ppm were not extracted for analysis. For the remaining plant samples, ^{14}C-residues were extracted repeatedly with MeOH:water (8:2, v:v), filtered, and concentrated to remove organic solvents. Extracted residues were then purified using a C_{18} SPE column, and the purified fractions were analyzed by 2D-TLC and RP-HPLC.

Solvent extraction released 40-109% of the TRR from [thiazol-^{14}C]-labeled samples and 40-99% of the TRR from [oxadiazin-^{14}C]-labeled samples. For a given crop matrix, the fractionation of ^{14}C-residues was similar for both ^{14}C-labels (Tables 32 and 33) and at different PBIs. Radioactivity in residual solids accounted for ≤0.012 ppm for all samples except wheat straw from the 30-day PBI.

Post-extraction solids from 30-day PBI wheat straw accounted for 0.106 and 0.064 ppm of radioactivity from the [thiazol-^{14}C] and [oxadiazin-^{14}C] samples, respectively. These solid fractions were further extracted by refluxing twice for 6 hrs. in MeOH:water (80:20, v:v), releasing an additional 42-45% of the TRR. These extracts were filtered and analyzed by 2D-TLC. The remaining solids from the [thiazol-^{14}C] sample were then refluxed in water:MeOH (80:20, v:v) for ~6 hrs, releasing an additional 16.3% of the TRR. ^{14}C-Residues in the final solid fractions from both wheat straw samples accounted for ≤0.045 ppm.

Table 32. Fractionation of ^{14}C-residues in RACs harvested from crops grown in soil treated with [thiazol-2-^{14}C]thiamethoxam at ~100 g ai/ha (~0.4x maximum seasonal rate).

Samples/ fraction	30-day PBI		120-day PBI		365-day PBI	
	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]
Mustard/spinach leaves [c]	--	0.019	--	0.015	--	0.017
MeOH/water [d]	100.9	0.019	101.5	0.015	76.9	0.013
solids	11.6	0.002	12.4	0.002	7.3	0.001
Turnip tops	--	0.051	--	0.014	--	0.026
MeOH/water	89.5	0.046	109.0	0.015	78.6	0.020
solids	18.0	0.009	16.5	0.002	13.5	0.004
25% wheat forage	--	0.036	--	0.018	--	0.015
MeOH/water	88.4	0.032	68.4	0.012	64.6	0.010
solids	18.4	0.007	21.4	0.004	20.7	0.003
50% wheat forage	--	0.036	--	0.008	--	0.021
MeOH/water	60.7	0.022	NA [e]		77.1	0.016
solids	23.1	0.008	NA		17.6	0.004
Wheat grain	--	0.019	--	0.006	--	0.009
MeOH/water	61.9	0.012	NA		NA	
solids	64.9	0.012	NA		NA	
Wheat straw	--	0.169	--	0.026	--	0.050
MeOH/water	39.6	0.067	63.2	0.016	43.5	0.022
solids [f]	63.0	0.106	43.9	0.011	47.0	0.024
MeOH:water	41.5	0.044	NA		NA	
Water:MeOH	16.3	0.017	NA		NA	
solids	26.6	0.045	NA		NA	

[a] % TRR values were not corrected for recovery.
[b] PPM values are expressed in [^{14}C]thiamethoxam equivalents.
[c] The representative leafy vegetable was mustard at the 30- and 120-day PBIs, and spinach at the 365-day PBI.
[d] MeOH/water fractions were purified using a SPE C_{18} column and analyzed by RP-HPLC and 2D-TLC.
[e] NA = Not analyzed.
[f] Wheat straw solids (30-day PBI) were further extracted by sequential refluxing in MeOH:water at 80:20 and 20:80 (v/v).

Table 33. Fractionation of ^{14}C-residues in RACs harvested from crops grown in soil treated with [oxadiazin-4-^{14}C]thiamethoxam at ~100 g ai/ha (~0.4x maximum seasonal rate).

Samples/ fraction	30-day PBI		120-day PBI		365-day PBI	
	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]
Mustard/spinach leaves [c]	--	0.016	--	0.023	--	0.012
MeOH/water [d]	94.2	0.015	97.2	0.022	85.8	0.010
solids	13.3	0.002	7.7	0.002	6.3	0.001
Turnip tops	--	0.045	--	0.016	--	0.027
MeOH/water	97.2	0.044	98.5	0.016	76.9	0.021
solids	15.7	0.007	16.0	0.003	11.4	0.003
25% wheat forage	--	0.022	--	0.024	--	0.015
MeOH/water	76.6	0.017	80.3	0.019	68.3	0.010
solids	18.4	0.004	17.7	0.004	16.5	0.002
50% wheat forage	--	0.031	--	0.010	--	0.029
MeOH/water	77.8	0.024	72.7	0.007	74.2	0.022
solids	17.1	0.005	22.6	0.002	16.2	0.005
Wheat grain	--	0.017	--	0.007	--	0.010
MeOH/water	78.9	0.013	NA [e]		48.5	0.005
solids	54.7	0.009	NA		52.5	0.005
Wheat straw	--	0.113	--	0.021	--	0.045
MeOH/water	40.4	0.046	53.0	0.011	44.4	0.020
solids [f]	56.6	0.064	45.3	0.010	58.4	0.026
MeOH:water	44.5	0.029	NA		NA	
solids	31.0	0.035	NA		NA	

[a] %TRR values were not corrected for recovery.
[b] PPM values are expressed in [^{14}C]thiamethoxam equivalents.
[c] The representative leafy vegetable was mustard at the 30- and 120-day PBIs, and spinach at the 365-day PBI.
[d] MeOH/water fractions were purified using a SPE C_{18} column and analyzed by RP-HPLC and 2D-TLC.
[e] NA = Not analyzed.
[f] Wheat straw solids (30-day PBI) were further extracted by refluxing in MeOH:water at 80:20 (v/v).

In addition to the plant sample analyses, soil samples were extracted with ACN, releasing 37.1-45.3% of the TRR from samples collected immediately following application and at each planting interval. Each of these ACN extracts was analyzed by 2D-TLC.

Characterization and identification of residues

^{14}C-Residues in purified MeOH/water extracts were analyzed and quantified by 2D-TLC using silica gel plates with methylethyl ketone:ACN (80:20) and EtOAc:PrOH:water (65:23:12), and

by reverse-phase HPLC using a C_{18} column with a linear gradient of 0.1% acetic acid in water to ACN. For the TLC analyses, reference compounds were detected using UV light and ^{14}C-residues were detected and quantified using an AMBIS radioanalytic imaging system. For the HPLC analyses, reference compounds were detected using a UV detector (254 nm) and ^{14}C-residues were detected using an in-line radioactivity monitor and by LSC of collected fractions. A total of 15 reference standards, including parent, were used for comparison. ^{14}C-Residues were identified by co-chromatography using RP-HPLC and 2D-TLC. The characterization and identification of ^{14}C-residues in rotational crops grown in soil treated with [^{14}C]thiamethoxam at ~100 g ai/ha (0.4x) are summarized in Tables 34 through 37.

The metabolic profile of ^{14}C-residues in rotational crops was qualitatively and quantitatively similar for the two ^{14}C-labels, with the exception of the [thiazol-^{14}C] specific metabolite CGA-359683. This minor metabolite was detected at various PBIs in mustard, spinach, turnip tops, and wheat forage and accounted for 5.6-10.7% TRR (0.001-0.004 ppm). Although there were quantitative differences, the metabolic profile was also qualitatively similar over time for the various rotational crops. The principal residues identified in rotational crops include parent and the metabolites CGA-322704 and CGA-265307.

Thiamethoxam was detected in each commodity except wheat straw, with levels being generally highest in turnip tops and leafy greens and at the shorter PBIs. In mustard and turnip tops, thiamethoxam accounted for 23.3-39.3% TRR (0.004-0.020 ppm) from the 30-day PBI and 32.2-58.2% TRR (0.006-0.008 ppm) from the 120-day PBI. By the 365-day PBI, residues of thiamethoxam declined to ≤3.3% TRR (≤0.001 ppm) in spinach and 26.3-31.2% TRR (≤0.008 ppm) in turnip tops. Thiamethoxam was detected at lower levels in wheat forage (4.8-14.2% TRR, ≤0.004 ppm) and grain (15.8% TRR, 0.003 ppm) from the 30-day PBI and in wheat forage (9.0-14.8% TRR, ≤0.004 ppm) from the 120-day PBI. By the 365-day PBI, thiamethoxam was not detected in any wheat commodities.

The metabolite CGA-322704 was also detected in each commodity (except grain) at various PBIs. For the 30-day PBI, CGA-322704 accounted for 8.0-12.6% TRR (0.001-0.006 ppm) in mustard and turnip tops and 10.0-35.2% TRR (0.002-0.020 ppm) in wheat forage and straw. At the later PBIs, the relative level of CGA-322704 increased in mustard/spinach and turnip tops to 9.1-47.8% TRR, although the actual residue levels (0.002-0.006 ppm) remained the same. For wheat forage and straw, CGA-322704 residues declined slightly to 6.4-19.2% TRR (0.002-0.005 ppm) at the 120- and 365-day PBIs.

The metabolite CGA-265307 accounted for a substantial portion of the residues in wheat straw although it was not detected in turnips at any PBI and was a minor component of the residues (2.6-14.6% TRR, ≤0.003 ppm) in mustard, spinach, and wheat forage and grain at various PBIs. For wheat straw from the 30-day PBI, CGA-265307 accounted for 26.0-32.5% TRR (0.030-0.055 ppm); levels of CGA-265307 subsequently declined in straw to 14.8-19.4% TRR (0.004 ppm) at the 120-day PBI and 5.1-11.7% TRR (0.003-0.005 ppm) at the 365-day PBI.

Low levels (1.8-19.6% TRR, ≤0.004 ppm) of CGA-322704-hydroxylamine-glucoside were also detected in each commodity from the 30-day PBI. However, this metabolite was not detected in any commodity from the 120-day PBI, and accounted for 10.6% TRR (0.002 ppm) in wheat forage from the 365-day PBI. Other minor metabolites identified in rotational crops included CGA-353968 and its degradate desmethyl-CGA-353968. CGA-353968 was detected at 2.6-14.6 % TRR (0.001-0.004 ppm) at various PBIs in each commodity except wheat grain. Desmethyl-CGA-353968 was only detected in wheat forage and straw at the 30- and 365-day PBIs and accounted for 3.0-6.9% TRR (0.001-0.007 ppm). In addition, solvent reflux of the 30-day [thiazol-^{14}C] straw sample released trace amounts of the ketone metabolite CGA-355190 (1.2% TRR, 0.002 ppm).

Although quantitative data for ^{14}C-residues in soil were not provided, the petitioner provided copies of 2D-TLC chromatograms from the analysis of ACN soil extracts. For both ^{14}C-labels, thiamethoxam was the only ^{14}C-residue detected in extracts of soil sampled immediately after application and at the 30-day PBI. Parent was also the principal component in soil extracts from the 120- and 365-day PBI; however, there was also a major spot of radioactivity that co-chromatographed with CGA-322704 and a minor spot of radioactivity that co-chromatographed with CGA-355190. Apparent levels of these metabolites were also higher at the 365-day PBI than at the 120-day PBI.

Based on these data, the petitioner has proposed that metabolism of thiamethoxam in rotational crops and soil primarily involves the opening of the oxadiazin ring to form CGA-322704, which is then either N-demethylated to yield CGA-265307 or N-hydroxylated and conjugated to form a glucoside of CGA-322704. A secondary pathway involves oxidation of the oxadiazin ring to form a ketone (CGA-355190), with subsequent ring opening to form CGA-353968. CGA-353968 is then demethylated to form desmethyl-CGA-353968, which undergoes oxidative bridge cleavage to form the thiazole ring metabolite CGA-359683.

Table 34. Summary of the characterization/identification of radioactive residues in RACs from rotational crops planted **30 days** following application of [thiazol-^{14}C]thiamethoxam at ~100 g ai/ha (~0.4x maximum seasonal rate).

Metabolite/ fraction	Mustard leaves (TRR = 0.019 ppm)		Turnip Tops (TRR = 0.051 ppm)		25% Wheat forage (TRR = 0.036 ppm)		50% Wheat forage (TRR = 0.036 ppm)		Wheat straw (TRR =0.169 ppm)		Wheat grain (TRR = 0.019 ppm)	
	%TRR	ppm [a]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Thiamethoxam	23.3	0.004	39.3	0.020	9.9	0.004	4.8	0.002	-- [b]	--	--	--
CGA-322704	10.5	0.002	12.6	0.006	18.5	0.007	11.6	0.004	10.0 [c]	0.017	--	--
CGA-322704-hydroxyl amine-glucoside	14.3	0.003	--	--	5.6	0.002	5.5	0.002	--	--	--	--
CGA-265307	3.1	0.001	--	--	2.6	0.001	5.5	0.002	32.5 [d]	0.055	14.6	0.003
CGA-355190	--	--	--	--	--	--	--	--	1.2 [e]	0.002	--	--
CGA-353968	--	--	3.7	0.002	6.9	0.002	--	--	2.6	0.004	--	--
Desmethyl-CGA-353968	--	--	--	--	--	--	3.6	0.001	3.0 [f]	0.005	--	--
CGA-359683	8.5	0.002	7.2	0.004	--	--	5.6	0.002	--	--	--	--
Total identified	**59.7**	**0.012**	**62.8**	**0.032**	**43.5**	**0.016**	**36.6**	**0.013**	**49.3**	**0.083**	**14.6**	**0.003**
Polar cluster I	33.2	0.006	19.1	0.010	37.9	0.014	19.0	0.007	12.4	0.021	47.3	0.009
Polar cluster II	8.0	0.001	7.6	0.004	7.0	0.002	5.1	0.002	4.1	0.007	--	--
Total identified/ characterized	**100.9**	**0.019**	**89.5**	**0.046**	**88.4**	**0.032**	**60.7**	**0.022**	**65.8**	**0.111**	**61.9**	**0.012**
Residual solids	11.6	0.002	18.0	0.009	18.4	0.007	23.1	0.008	26.6	0.045	64.9	0.012

[a] Expressed in [^{14}C]thiamethoxam equivalents.
[b] -- = not detected.
[c] includes CGA-322704 identified in polar cluster I and in reflux extract of initial solids.
[d] includes CGA-265307 identified in polar clusters I and II and in reflux extract of initial solids.
[e] Identified only in the reflux extract.
[f] includes desmethyl-CGA-353968 identified in reflux extract of initial solids.

Table 35. Summary of the characterization/identification of radioactive residues in RACs from rotational crops planted **30 days** following application of [oxadiazin-^{14}C]thiamethoxam at ~100 g ai/ha (~0.4x maximum seasonal rate).

Metabolite/ fraction	Mustard leaves (TRR = 0.016 ppm)		Turnip Tops (TRR = 0.045 ppm)		25% Wheat forage (TRR = 0.022 ppm)		50% Wheat forage (TRR = 0.031ppm)		Wheat straw (TRR =0.113 ppm)		Wheat grain (TRR = 0.017 ppm)	
	%TRR	ppm [a]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Thiamethoxam	30.6	0.005	26.4	0.012	5.4	0.001	14.2	0.004	-- [b]	--	15.8	0.003
CGA-322704	8.0	0.001	10.9	0.005	12.4	0.003	35.2	0.011	17.7 [c]	0.020	--	--
CGA-322704-hydroxyl amine-glucoside	12.3	0.002	9.3	0.004	--	--	--	--	1.8	0.002	19.6	0.003
CGA-265307	--	--	--	--	4.5	0.001	--	--	26.0 [d]	0.030	12.6	0.002
CGA-353968	4.4	0.001	3.3	0.002	7.3	0.002	8.7	0.003	--	--	--	--
Desmethyl-CGA-353968	--	--	--	--	--	--	--	--	6.2 [e]	0.007	--	--
Total identified	**55.3**	**0.009**	**49.9**	**0.023**	**29.6**	**0.007**	**58.1**	**0.018**	**51.7**	**0.059**	**48**	**0.008**
Unknown	--	--	--	--	8.6	0.002	--	--	--	---	--	--
Polar cluster I	32.0	0.005	31.9	0.014	23.1	0.005	19.8	0.006	9.7	0.011	30.8	0.005
Polar cluster II	6.7	0.001	15.4	0.007	15.1	0.003	--	--	4.4	0.005	--	--
Total identified/ characterized	**94.0**	**0.015**	**97.2**	**0.044**	**76.4**	**0.017**	**77.9**	**0.024**	**65.8**	**0.075**	**78.8**	**0.013**
Residual solids	13.3	0.002	15.7	0.007	18.4	0.004	17.1	0.005	31.0	0.035	54.7	0.009

[a] Expressed in [^{14}C]thiamethoxam equivalents.
[b] -- = not detected.
[c] includes CGA-322704 identified in polar cluster I and in reflux extract of initial solids.
[d] includes CGA-265307 identified in reflux extract of initial solids.
[e] Identified only in the reflux extract.
[f] includes desmethyl-CGA-353968 identified in reflux extract of initial solids.

Table 36. Summary of the characterization/identification of radioactive residues in RACs from rotational crops planted **120 days** following application of [thiazol-^{14}C] or [oxadiazin-^{14}C]thiamethoxam at ~100 g ai/ha (~0.4x maximum seasonal rate).

Metabolite/ fraction	Mustard leaves		Turnip Tops		25% Wheat forage [a]		Wheat straw	
	%TRR	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm
[Thiazol-^{14}C]Thiamethoxam								
Thiamethoxam	55.8	0.008	58.2	0.008	9.0	0.002	-- [c]	--
CGA-322704	19.3	0.003	40.2	0.006	16.1	0.003	9.9	0.003
CGA-265307	--	--	--	--	--	--	14.8	0.004
CGA-353968	--	--	--	--	--	--	5.9	0.002
CGA-359683	--	--	10.7	0.002	--	--	--	--
Total identified	**75.1**	**0.011**	**109.1**	**0.016**	**25.1**	**0.01**	**30.6**	**0.009**
Polar cluster I	26.4	0.004	--	--	26.6	0.005	20.2	0.005
Polar cluster II	--	--	--	--	16.6	0.003	12.4	0.003
Total identified/ characterized	**101.5**	**0.015**	**109.1**	**0.016**	**68.3**	**0.013**	**63.2**	**0.017**
Residual solids	12.4	0.002	16.5	0.002	21.4	0.004	43.9	0.011
[Oxadiazin-^{14}C]Thiamethoxam								
Thiamethoxam	32.2	0.007	38.6	0.006	14.8	0.004	--	-
CGA-322704	9.1	0.002	29.6	0.005	19.2	0.004	18.9	0.004
CGA-265307	--	--	--	--	--	--	19.4	0.004
CGA-353968	14.6	0.003	7.7	0.001	5.7	0.001	--	--
Total identified	**55.9**	**0.012**	**75.9**	**0.012**	**39.7**	**0.01**	**38.3**	**0.008**
Polar cluster I	28.9	0.007	15.4	0.002	26.5	0.006	14.7	0.003
Polar cluster II	12.4	0.003	7.3	0.001	14.0	0.003	--	--
Total identified/ characterized	**97.2**	**0.022**	**98.6**	**0.015**	**80.2**	**0.018**	**53.0**	**0.011**
Residual solids	7.7	0.002	16.0	0.003	17.7	0.004	45.3	0.010

[a] Results from the analysis of 50% mature forage were similar results from 25% mature forage.
[b] Expressed in [^{14}C]thiamethoxam equivalents.
[c] -- = Not detected.

Table 37. Summary of the characterization/identification of radioactive residues in RACs from rotational crops planted **365 days** following application of [thiazol-^{14}C] or [oxadiazin-^{14}C]thiamethoxam at ~100 g ai/ha (~0.4x maximum seasonal rate).

Metabolite/ fraction	Spinach leaves		Turnip Tops		25% Wheat forage [a]		Wheat straw	
	%TRR	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm
[Thiazol-^{14}C]Thiamethoxam								
Thiamethoxam	3.3	0.001	26.3	0.007	-- [c]	--	--	--
CGA-322704	33.8	0.006	17.0	0.004	10.7	0.002	6.4	0.003
CGA-265307	8.8	0.002	--	--	5.2	0.001	5.7	0.003
CGA-353968	6.3	0.001	10.1	0.003	6.8	0.001	--	--
Desmethyl-CGA-353968	--	--	--	--	6.9	0.001	5.1	0.003
CGA-359683	5.7	0.001	--	--	10.4	0.002	--	--
Total identified	**57.9**	**0.011**	**53.4**	**0.014**	**40**	**0.01**	**17.2**	**0.009**
Polar cluster I	19.0	0.003	25.1	0.006	16.3	0.002	14.7	0.008
Polar cluster II	--	--	--	--	8.2	0.001	11.6	0.006
Total identified/ characterized	**76.9**	**0.014**	**78.5**	**0.020**	**64.5**	**0.01**	**43.5**	**0.023**
Residual solids	7.3	0.001	13.5	0.004	20.7	0.003	47.0	0.024
[Oxadiazin-^{14}C]Thiamethoxam								
Thiamethoxam	--	--	31.2	0.008	--	--	--	--
CGA-322704	47.8	0.006	15.1	0.004	17.5	0.003	11.9	0.005
CGA-265307	7.2	0.001	--	--	--	--	11.7	0.005
CGA-322704-OH amine-glucoside	--	--	--	--	10.6	0.002	--	--
Total identified	**55**	**0.007**	**46.3**	**0.012**	**28.1**	**0.01**	**23.6**	**0.01**
Polar cluster I	30.8	0.004	18.2	0.005	27.4	0.004	20.8	0.009
Polar cluster II	--	--	12.3	0.003	12.8	0.002	--	--
Total identified/ characterized	**85.8**	**0.011**	**76.8**	**0.02**	**68.3**	**0.011**	**44.4**	**0.019**
Residual solids	6.3	0.001	11.4	0.003	16.5	0.002	58.4	0.026

[a] Results from the analysis of 50% mature forage were similar results from 25% mature forage.
[b] Expressed in [^{14}C]thiamethoxam equivalents.
[c] -- = Not detected.

Summary

Although this study was not conducted at 1x the maximum seasonal rate, this confined rotational crop study is acceptable as ^{14}C-residues were sufficiently identified/characterized and the available data allow HED to conclude that limited field rotational crop studies are necessary to support the proposed 120-day PBI for rotational crops.

Following a soil application of [^{14}C]thiamethoxam at ~100 g ai/A (~0.4x), radioactive residues were generally low (≤0.05 ppm) in plant samples, with the exception of wheat straw. Radioactive residues were lowest in turnip roots (<0.01 ppm) followed by wheat grain (0.006-0.019 ppm) and mustard/spinach leaves (0.012-0.023 ppm). Radioactive residues were 0.008-0.036 ppm in wheat forage and 0.021-0.169 ppm in straw. For each commodity, ^{14}C-residues were generally highest at the 30-day PBI and lowest at the 120-day PBI. ^{14}C-Residues increased slightly in most commodities between the 120- and 365-day PBIs.

Radioactive residues in rotational crops were adequately identified or characterized. The principal metabolites identified in rotational crops were parent, CGA-322704, and CGA-265307. At the 30-day PBI, thiamethoxam was detected in each commodity except wheat straw, with levels being generally highest in turnip tops and mustard (23.3-39.3% TRR, 0.004-0.020 ppm) and lowest in wheat forage and grain (4.8-15.8% TRR, ≤0.004 pm). By the 365-day PBI, residues of thiamethoxam declined to ≤3.3% TRR (≤0.001 ppm) in spinach and 26.3-31.2% TRR (≤0.008 ppm) in turnip tops, and were not detected in any wheat commodities. The metabolite CGA-322704 was also detected in each commodity (except grain) at various PBIs and accounted for 6.4-47.8% TRR (0.001-0.020 ppm). Metabolite CGA-265307 accounted for a substantial portion of the residues in wheat straw (5.1-32.5% TRR, 0.003-0.055 ppm), but was not detected in turnip tops and was a minor component of the residues in mustard, spinach, and wheat forage and grain (2.6-14.6% TRR, ≤0.003 ppm). Other minor metabolites identified in rotational crops included CGA-322704-hydroxylamine-glucoside (1.8-19.6% TRR, ≤0.004 ppm), CGA-353968 (2.6-14.6 % TRR, ≤0.004 ppm), desmethyl-CGA-353968 (3.0-6.9% TRR, ≤0.007 ppm), CGA-359683 (5.6-10.7% TRR; ≤0.004) and CGA-355190 (1.2% TRR, 0.002 ppm).

Confined Rotational Crop Study II. In addition to the above study, the petitioner also submitted data from two related studies depicting the accumulation of ^{14}C-residues in confined rotational crops following an application of either [thiazol-2-^{14}C] or [oxadiazin-4-^{14}C]thiamethoxam at ~200 g ai/ha (0.18 lb ai/A; ~0.8x) to the soil surface. The in-life and analytical phases of these studies were conducted by Novartis at their research facilities in St. Aubin and Basel, Switzerland. Results from these studies are reported in:

44715116 Sandmeier, P. (1997) Outdoor Confined Study on Rotational Crops After Bareground Application of (Thiazol-2-^{14}C) CGA-293343: Lab Project Number: CMR 16/97: 766-97: 95PSA43. Unpublished study prepared by Novartis Crop Protection, Inc. 141 p.

44715117 Sandmeier, P. (1997) Outdoor Confined Study on Rotational Crops After Bareground Application of (Oxadiazin-4-^{14}C) CGA-293343: Lab Project Number: CMR 20/97: 765-97: 95PSA42PR1. Unpublished study prepared by Novartis Crop Protection, Inc. 151 p.

The [thiazol-2-^{14}C]- and [oxadiazin-4-^{14}C]-labeled test substances were diluted with non-labeled thiamethoxam and formulated as a 25% WP for application. The [^{14}C]thiamethoxam in both formulations had a final specific activity of 27.03 μCi/mg (60,006 dpm/μg) and a radiochemical purity of ≥96%.

Each formulated test substance was diluted with water and applied as a broadcast spray to an outdoor plot of loam soil (47% sand, 32% silt, 21% clay, and 3.1% organic matter; pH 7.2; and CEC of 17.1 meq/100 g) at a target rate of 200 g ai/ha. Actual application rates were 207 and 200 g ai/ha for the [thiazol-^{14}C] and [oxadiazin-^{14}C] test substances, respectively, equivalent to 0.18 lb ai/A (~0.8x). The control and treated plots were each subdivided into four subplots and remained fallow until the first set of rotational crops was planted.

Lettuce, radishes, and spring wheat were planted as representative rotational crops at approximately 1, 4, and 12 months following the soil application. In addition, winter wheat was also planted at 6 months posttreatment. Each subplot was planted with a single rotational crop, and following harvest, was replanted with a different rotational crop for a subsequent PBI. Of the four subplots per treatment, two were planted twice and two were planted with three different rotational crops in succession. *[HED notes that although guidelines (OPPTS 860.1850) allow for the planting and harvest of a primary crop, individual plots should not be used for a succession of rotational crops].* The crops received water, fertilizer, and maintenance pesticides as necessary; adequate information pertaining to the growing conditions was provided.

A single sample of each commodity was collected at each PBI from the control and treated plots. Lettuce and radishes were harvested at maturity (60-63 DAP), and the radishes were separated into tops and roots. Spring wheat was harvested for forage at 50% crop maturity (60-112 DAP) and for straw, grain and husks (95-146 DAP) at maturity. For the winter wheat, forage was collected twice, once in the fall (70 DAP) and again in the spring at 50% maturity (245 DAP); grain, straw, and husks were collected at maturity (294 DAP). In addition, soil core samples (0-30 cm) were collected prior to and immediately following treatment and at each planting interval. Soil core samples were further separated into 0-10, 10-20, and 20-30 cm segments. Samples were shipped within 24 hours to the analytical laboratory where they were stored at ≤-18 C until analysis. Samples of grain, husks, and straw were air dried at room temperature for at least one week prior to processing for analysis.

Total radioactive residues (TRR)

Crop and soil samples were ground with dry ice and radioassayed in triplicate by LSC following combustion. The LOQ for the combustion was 0.001 ppm for lettuce, radish tops and roots, wheat forage, and soil, and 0.004 ppm for wheat grain, straw, and husks. The TRRs in rotational crop commodities and soil samples are presented in Table 38.

Table 38. Total radioactive residues found in/on representative rotational crops grown in a loam soil treated with [^{14}C]thiamethoxam at ~200 g ai/ha (~0.8 x the maximum proposed seasonal rate).

Crop/commodity	Plant-back Interval (days)	Sampling interval [a]		Total radioactive residues (ppm) [b]	
		DAT	DAP	[Thiazol-^{14}C]	[Oxadiazin-^{14}C]
Lettuce Leaves	29	89	60	0.035	0.034
	119	180	61	0.013	0.012
	362	425	63	0.004	0.008
Radish Tops	29	89	60	0.116	0.077
	119	180	61	0.011	0.011
	362	425	63	0.009	0.008
Radish Roots	29	89	60	0.007	0.005
	119	180	61	0.002	0.002
	362	425	63	0.003	0.002
Wheat forage [c]	29	89	60	0.112	0.067
	104	180	76	0.030	0.056
	180	250	70	0.014	0.023
		425	245	0.009	0.010
	362	474	112	0.019	0.035
Wheat straw	29	124	95	0.753	0.520
	104	250	146	0.172	0.233
	180	474	294	0.051	0.057
	362	492	130	0.082	0.080
Wheat husks	29	124	95	0.365	0.390
	104	250	146	0.131	0.180
	180	474	294	0.052	0.069
	362	492	130	0.058	0.072
Wheat grain	29	124	95	0.029	0.020
	104	250	146	0.147	0.085
	180	474	294	0.005	0.006
	362	492	130	0.004	0.007
Soil (0-10 cm)	NA	0	NA	0.186	0.165
	NA	29	NA	0.143	0.147
	NA	119	NA	0.086	0.079
	NA	180	NA	0.055	0.074
	NA	362	NA	0.041	0.050

[a] Crop sampling intervals are expressed in terms of days after soil treatment (DAT) and days after crop planting (DAP).

[b] Data are expressed in [^{14}C]thiamethoxam equivalents and are the average of triplicate analyses.

[c] Wheat forage samples were collected at 50% maturity with the exception of the fall cutting of forage (250 DAT) from the 180-day PBI.

Levels of ^{14}C-residues resulting from application of the two ^{14}C-labels were similar for each crop matrix, and with a few exceptions, ^{14}C-residues in each commodity declined steadily at succeeding PBIs. At each PBI, ^{14}C-residues were lowest in radish roots (<0.01 ppm) and were also generally low (<0.03 ppm) in wheat grain, with the exception of ^{14}C-residues in grain from the 104-day PBI (0.085-0.147 ppm) which the petitioner attributed to poor growing conditions. The highest ^{14}C-residues at each PBI occurred in wheat straw (0.051-0.753 ppm) and husks (0.052-0.390 ppm).

The level and pattern of decline in ^{14}C-residues in soil was similar for the two ^{14}C-labels. On the day of application, ^{14}C-residues were 0.186 and 0.165 ppm in the top 10 cm of soil from the [thiazol-^{14}C] and [oxadiazin-^{14}C]treated plots, respectively. ^{14}C-Residues in soil declined steadily to 0.041-0.050 ppm in the top soil layer by 362 DAT.

Storage stability data

Except for several samples, plant samples for this confined rotational crop study were stored at ≤18 C for ≤2.8 months prior to extraction for analysis. Samples of lettuce (119-day PBI) and wheat forage (104-day PBI) from both ^{14}C-labels and radish tops (119-day PBI) from the [thiazol-^{14}C]label were all stored frozen for 5 months prior to extraction for analysis.

To support the stability of ^{14}C-residues during storage, the petitioner reextracted and analyzed [thiazol-^{14}C]-treated samples of lettuce and radish tops and [oxadiazin-^{14}C]-treated samples of wheat grain and straw from the 29-day PBI. These samples, which were originally extracted for analysis after 11 days of frozen storage, were reextracted for analysis at the end of the study, after an additional 17-18 months of storage. The petitioner presented example chromatograms and quantitative data for metabolites showing that ^{14}C-residues were stable over the ~18 months of storage. No additional storage stability data are required to support this confined rotational crop study.

Extraction and hydrolysis of residues

Plant samples with TRR values of ≤0.01 ppm were not extracted for analysis. For the remaining plant samples, ^{14}C-residues were extracted repeatedly with MeOH:water (8:2, v:v), filtered, combined and concentrated to remove organic solvents. The methanolic extracts from 29-day PBI samples of lettuce, radish tops, and wheat forage and straw were then directly analyzed by 2D-TLC. Methanolic extracts from the remaining plant samples were first partitioned between DCM and water prior to TLC analysis. In addition, DCM fractions from several wheat straw and grain samples were also cleaned up using a C_{18} column eluted with water and MeOH prior to analysis.

Solvent extracted solids from most samples containing ≥0.01 ppm of radioactivity were extracted further by Soxhlet extraction with MeOH:water (8:2, v:v). Soxhlet-extracted residual

solids from wheat straw (29- and 104-day PBIs) were further extracted using accelerated solvent extraction (ASE) with 1-propanol (PrOH):water (8:2, v/v) at 170 C and 1450 psi. Solubilized ^{14}C-residues were analyzed by 2D-TLC and the remaining solids were hydrolyzed by refluxing in 2N NaOH for 3 hours. The resulting hydrolysate was then acidified and concentrated to precipitate lignin.

Soxhlet-extracted solids from wheat grain (104-day PBI) samples were further extracted with 0.05N NaOH to isolate proteins and the remaining solids were then hydrolyzed by refluxing in 1N HCl overnight. The resulting acid hydrolysate was neutralized and partitioned with DCM. ^{14}C-Residues in the aqueous phase were then derivatized by refluxing with phenylhydrazine-hydrochloride, cooled, and filtered. The resulting glucosazone was filtered, redissolved in MeOH/water and recrystallized. In addition to the osazone derivation, ^{14}C-residues in the aqueous phase were also fractionated using a series of anion and cation exchange chromatography columns.

The fractionation and distribution of ^{14}C-residues in each crop matrix were similar for both ^{14}C-labels and at the different PBIs (Tables 39 and 40). The petitioner also analyzed wheat husk samples from each PBI; however, these samples are not presented in this report as husks are not a regulated RAC and the ^{14}C-residue profile in husks was similar to the profile in wheat straw.

The initial methanolic extraction released 75-92% of the TRR from lettuce, radish tops and wheat forage samples, 50-65% of the TRR from wheat straw, and 10-50% of the TRR from wheat grain. Subsequent Soxhlet extraction released only an additional 1.5-11.5% of the TRR. With the exceptions of 29-day PBI wheat straw and 104-day PBI wheat straw and grain samples, radioactivity in residual solids amounted to 0.002-0.036 ppm and were not further analyzed. For wheat straw from the 29- and 104-day PBIs, ASE extraction solubilized an additional 15-18% of the TRR, which was then analyzed by 2D-TLC. For 104-day PBI wheat grain samples, 0.05N NaOH extraction released 17-18% of the TRR, of which 9-10% TRR precipitated out with the protein fraction, and acid hydrolysis released 56-57% of the TRR. Radioactivity in residual solids from wheat grain and straw amounted to $\leq$0.04 ppm.

Table 39. Fractionation and identification of ^{14}C-residues in RACs harvested from crops grown in soil treated with [thiazol-2-^{14}C]thiamethoxam at ~200 g ai/ha (~0.8x maximum seasonal rate).

Fraction	% TRR [a]	ppm	Characterization/Identification
29-day PBI, Lettuce (0.035 ppm)			
MeOH:H_2O	78.3	0.027	2D-TLC analysis: Thiamethoxam 21.3% TRR 0.008 ppm CGA-322704 12.1% TRR 0.004 ppm NOA-407475 4.5% TRR 0.002 ppm NOA-421275 8.2% TRR 0.003 ppm Unknown + Unresolved 32.0% TRR 0.011 ppm each at ≤10.8% TRR ≤0.004 ppm Identity of isolated thiamethoxam and CGA-322704 was confirmed by reverse-phase TLC.
MeOH Soxhlet	1.5	<0.001	Not further analyzed.
Residual solids	17.3	0.006	
119-day PBI, Lettuce (0.013 ppm)			
MeOH:H_2O	76.6	0.010	Concentrated and partitioned between DCM and H_2O.
DCM	42.4	0.006	2D-TLC analysis: Thiamethoxam 18.4% TRR 0.002 ppm CGA-322704 15.9% TRR 0.002 ppm Unresolved 8.1% TRR 0.001 ppm
Aqueous	33.6	0.004	Not further analyzed.
Residual solids	24.9	0.003	
29-day PBI, Radish tops (0.116 ppm)			
MeOH:H_2O	80.1	0.093	2D-TLC analysis: Thiamethoxam 19.8% TRR 0.023 ppm CGA-322704 6.8% TRR 0.008 ppm NOA-407475 6.7% TRR 0.008 ppm NOA-421275 6.3% TRR 0.007 ppm CGA-265307 9.1% TRR 0.011 ppm CGA-353968 5.2%TRR 0.006 ppm Desmethyl-CGA-353968 2.0% TRR 0.002 ppm CGA-355190 0.9% TRR 0.001 ppm Unknowns + Unresolved 23.3% TRR 0.027 ppm each at ≤7.1% TRR ≤0.008 ppm Each metabolite (except 355190) was isolated and its Identity confirmed by reverse phase TLC with standards.
MeOH Soxhlet	1.6	0.002	Not further analyzed.
Residual solids	9.4	0.011	
119-day PBI, Radish tops (0.011 ppm)			
MeOH:H_2O	81.5	0.009	Not further analyzed.
Residual solids	23.1	0.003	

(continued; footnotes follow)

Table 39. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
29-day PBI, Wheat forage (0.112 ppm)			
MeOH:H_2O	77.7	0.087	2D-TLC analysis: Thiamethoxam 4.4% TRR 0.005ppm CGA-322704 10.0% TRR 0.011ppm NOA-407475 6.3% TRR 0.007 ppm NOA-421275 20.7% TRR 0.023 ppm CGA-265307 3.7% TRR 0.004 ppm Desmethyl-CGA-353968 1.2% TRR 0.001 ppm Unknowns + Unresolved 31.5% TRR 0.035 ppm each at ≤10.4% TRR ≤ 0.011ppm
MeOH Soxhlet	5.6	0.006	Not further analyzed.
Residual solids	9.4	0.011	
104-day PBI, Wheat forage (0.030 ppm)			
MeOH:H_2O	79.6	0.024	Concentrated and partitioned between DCM and H_2O.
DCM	23.6	0.007	2D-TLC analysis: CGA-322704 16.6% TRR 0.005 ppm CGA-265307 2.6% TRR <0.001 ppm Unknowns + Unresolved 4.4% TRR 0.001 ppm
Aqueous	57.7	0.017	2D-TLC analysis: CGA-322704 2.2% TRR <0.001 ppm NOA-407475 1.6% TRR <0.001 ppm NOA-421275 9.3% TRR 0.003 ppm CGA-265307 2.1% TRR <0.001 ppm Desmethyl-CGA-353968 2.0% TRR <0.001 ppm Unknown(s)+ Unresolved 40.4% TRR 0.012 ppm each at ≤16.3% TRR ≤0.005 ppm
Residual solids	NR	--	Soxhlet extracted with MeOH
MeOH Soxhlet	6.8	0.002	Not further analyzed.
Residual solids	19.6	0.006	
180-day PBI Wheat forage (0.014 ppm)			
MeOH:H_2O	74.7	0.010	Concentrated and partitioned between DCM and H_2O.
DCM	43.3	0.006	2D-TLC analysis: Thiamethoxam 1.6% TRR <0.001 ppm CGA-322704 31.2% TRR 0.004 ppm CGA-265307 4.1% TRR <0.001 ppm Known(s) + Unresolved 6.1% TRR <0.001 ppm
Aqueous	28.9	0.004	Not further analyzed.
Residual solids	25.8	0.004	

(continued; footnotes follow)

Table 39. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
362-day PBI Wheat forage (0.019 ppm)			
MeOH:H_2O	80.5	0.015	Concentrated and partitioned between DCM and H_2O.
DCM	11.7	0.002	2D-TLC analysis: CGA-322704 4.3% TRR <0.001 ppm CGA-265307 3.8% TRR < 0.001 ppm Unknown(s)+ Unresolved 3.9% TRR <0.001 ppm
Aqueous	64.3	0.012	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted with MeOH.
MeOH Soxhlet	5.3	0.001	Not further analyzed.
Residual solids	24.5	0.005	
29-day PBI Wheat straw (0.753 ppm)			
MeOH:H_2O	58.3	0.439	2D-TLC analysis: Thiamethoxam 5.1% TRR 0.038 ppm CGA-322704 5.8% TRR 0.044 ppm NOA-407475 3.0% TRR 0.023 ppm NOA-421275 8.7% TRR 0.066 ppm CGA-265307 5.1% TRR 0.038 ppm Desmethyl CGA-353968 3.1% TRR 0.023 ppm Unknowns+Unresolved 27.3% TRR 0.206 ppm each at ≤8.6% TRR ≤0.065 ppm Identity of isolated TLC fractions were confirmed by reverse phase TLC analysis with reference standards.
Residual solids	NR	--	Soxhlet extracted with MeOH.
MeOH Soxhlet	3.3	0.025	Not further analyzed.
Residual solids	30.2	0.227	ASE extraction with PrOH:H_2O at 170 C and 1450 psi..
PrOH:H_2O extract	16.0	0.120	Concentrated and resolubized in PrOH:H_2O, yielded fraction with 9.6% TRR (0.072 ppm). 2D-TLC analysis: CGA-322704 1.1% TRR 0.008 ppm CGA-265307 1.2% TRR 0.009 ppm Desmethyl CGA-353968 0.3% TRR 0.002 ppm Unknowns+Unresolved 7.0% TRR 0.052 ppm each at ≤2.4% TRR ≤0.018 ppm
Residual solids	NR	--	Hydrolyzed in boiling 2N NaOH
Hydrolysate	9.2	0.069	Acidified to pH 1, concentrated and centrifuged to precipitate lignin.
Precipitate	2.1	0.016	Radioactivity associated with lignin fraction.
Filtrate	4.9	0.036	Not further analyzed.
Residual solids	1.6	0.012	

(continued; footnotes follow)

Table 39. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
104-day PBI Wheat straw (0.172 ppm)			
MeOH:H_2O	50.2	0.086	Concentrated and partitioned between DCM and H_2O.
DCM	12.9	0.022	Purified by C_{18} column 2D-TLC analysis: Thiamethoxam 0.4% TRR 0.003 ppm CGA-322704 6.1% TRR 0.010 ppm CGA-265307 4.6% TRR 0.008 ppm Unknown+Unresolved 1.8% TRR 0.003 ppm
Aqueous	39.3	0.068	2D-TLC analysis: Thiamethoxam 3.7% TRR 0.006 ppm NOA-421275 4.8% TRR 0.008 ppm CGA-265307 3.0% TRR 0.005 ppm Desmethyl-CGA-353968 1.6% TRR 0.003 ppm Unknown(s)+ Unresolved 26.2% TRR 0.045 ppm each at ≤14.1% TRR 0.024 ppm
Residual solids	NR	--	Soxhlet extracted with MeOH.
MeOH Soxhlet	6.9	0.012	Not further analyzed.
Residual solids	43.5	0.075	ASE extraction with PrOH:H_2O at 170 C and 1450 psi..
PrOH:H_2O extract	17.7	0.030	Concentrated and resolubized in PrOH:H_2O, yielded fraction with 14.3% TRR (0.025 ppm). 2D-TLC analysis: CGA-322704 1.6% TRR 0.003 ppm CGA-265307/Desmethyl CGA-353968 2.3% TRR 0.004 ppm Unknown+Unresolved 10.1% TRR 0.017 ppm each at ≤4.1% TRR 0.007 ppm
Residual solids	21.4	0.037	Not further analyzed.
180-day PBI Wheat straw (0.051 ppm)			
MeOH:H_2O	57.1	0.029	Concentrated and partitioned between DCM and H_2O.
DCM	12.3	0.006	Cleaned up on a C18 column eluted with H_2O and MeOH.
Aqueous	1.4	<0.001	Not further analyzed.
MeOH	10.5	0.005	2D-TLC analysis: CGA-322704 3.8% TRR 0.002 ppm CGA-265307 3.4% TRR 0.002 ppm Unknown + Unresolved 3.3% TRR 0.002 ppm
Aqueous	42.9	0.022	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted in MeOH.
MeOH Soxhlet	4.8	0.002	Not further analyzed.
Residual solids	43.2	0.022	

(continued; footnotes follow)

Table 39. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
362-day PBI Wheat straw (0.082 ppm)			
MeOH:H_2O	55.1	0.045	Concentrated and partitioned between DCM and H_2O.
DCM	12.6	0.010	Cleaned up on a C18 column eluted with H_2O and MeOH. 2D-TLC analysis: CGA-322704 5.4% TRR 0.004 ppm CGA-265307 4.3% TRR 0.004 ppm Unknown + Unresolved 2.8% TRR 0.002 ppm
Aqueous	39.3	0.032	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted in MeOH.
MeOH Soxhlet	3.2	0.002	Not further analyzed.
Residual solids	44.1	0.036	
29-day Wheat grain (0.029 ppm)			
MeOH:H_2O	50.0	0.015	Concentrated and partitioned between DCM and H_2O.
DCM	13.6	0.004	2D-TLC analysis: Thiamethoxam 0.3% TRR <0.001 ppm CGA-322704 3.8% TRR 0.001 ppm CGA-265307 7.4% TRR 0.002 ppm Unknown + Unresolved 2.1% TRR <0.001 ppm
Aqueous	31.7	0.009	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted in MeOH.
MeOH Soxhlet	3.1	<0.001	Not further analyzed.
Residual solids	65.1	0.019	
104-day PBI Wheat grain (0.147 ppm)			
MeOH:H_2O	10.8	0.016	Concentrated and partitioned between DCM and H_2O.
DCM	2.6	0.004	Cleaned up on a C_{18} column (89.2% recovery) 2D-TLC analysis: CGA-322704 0.2% TRR <0.001 ppm CGA-265307 0.7% TRR 0.001 ppm Unknowns + Unresolved 2.0% TRR 0.003 ppm
Aqueous	6.3	0.009	2D-TLC analysis: CGA-265307 and Desmethyl CGA-353968 0.5% TRR <0.001 ppm Unknowns + Unresolved 5.8% TRR 0.009 ppm
Precipitate	1.5	0.002	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted in MeOH.
MeOH Soxhlet	1.9	0.003	Not further analyzed.
Residual solids	89.3	0.131	Extracted with 0.05N NaOH, neutralized, and proteins precipitated with EtOH.
Extract	8.9	0.013	Not further analyzed.
Precipitate	8.8	0.013	Radioactivity associated with protein fraction.

(continued; footnotes follow)

Table 39. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
Residual solids	NR	--	Hydrolyzed by refluxing overnight in 1 N HCl
Acid hydrolysate	55.9	0.082	Neutralized and partitioned with DCM
DCM	0.4	<0.001	Not further analyzed.
Aqueous	55.1	0.081	Derivatized, recrystallized, and filtered to yield glucosazone and filtrate fractions. An additional aliquot was further fractionated and purified by anion and cation exchange chromatography; however, no fractions suitable for TLC analysis were obtained.
Starch	16.8	0.025	Identified as being incorporated into starch.
Filtrate	37.2	0.055	Not further analyzed
Residual solids	12.0	0.017	Not further analyzed.

[a] Not corrected for recovery.

Table 40. Fractionation of ^{14}C-residues in RACs harvested from crops grown in soil treated with [oxadiazin-4-^{14}C]thiamethoxam at ~200 g ai/ha (1.8x maximum seasonal rate).

Fraction	% TRR [a]	ppm	Characterization/Identification
29-day PBI, Lettuce (0.034 ppm)			
MeOH:H_2O	92.2	0.031	2D-TLC analysis: Thiamethoxam 25.1% TRR 0.009 ppm CGA-322704 11.9% TRR 0.004 ppm NOA-407475 5.0% TRR 0.002 ppm CGA-382191 4.8% TRR 0.002 ppm NOA-421275 5.4% TRR 0.002 ppm NOA-405217 11.2% TRR 0.004 ppm Unknown(s)+ Unresolved 29.0% TRR 0.010 ppm
MeOH Soxhlet	1.9	<0.001	Not further analyzed.
Residual solids	14.5	0.005	
119-day PBI, Lettuce (0.012 ppm)			
MeOH:H_2O	85.2	0.010	Concentrated and partitioned between DCM and H_2O.
DCM	34.3	0.004	2D-TLC analysis: Thiamethoxam 12.2% TRR 0.002 ppm CGA-322704 13.7% TRR 0.002 ppm Unknown I_{20} 2.6% TRR <0.001 ppm Unresolved 5.8% TRR <0.001 ppm
Aqueous	48.4	0.006	Not further analyzed.
Residual solids	19.2	0.002	
29-day PBI, Radish tops (0.077 ppm)			
MeOH:H_2O	94.8	0.073	2D-TLC analysis: Thiamethoxam 24.4% TRR 0.019 ppm CGA-322704 15.5% TRR 0.012 ppm NOA-407475 9.2% TRR 0.007 ppm CGA-382191 6.4% TRR 0.005 ppm NOA-421275 7.5% TRR 0.006 ppm CGA-265307 8.9% TRR 0.007 ppm NOA-405217 2.8% TRR 0.002 ppm CGA-355190 1.3% TRR 0.001 ppm Desmethyl-CGA-353968 1.8% TRR 0.001 ppm Unknown(s)+ Unresolved 17.1% TRR 0.013 ppm
MeOH Soxhlet	2.2	0.002	Not further analyzed.
Residual solids	7.8	0.006	
119-day PBI, Radish tops (0.011 ppm)			
MeOH:H_2O	90.1	0.010	Concentrated and partitioned between DCM and H_2O.
DCM	22.9	0.003	2D-TLC analysis: Thiamethoxam 9.7% TRR 0.001 ppm CGA-322704 6.5% TRR <0.001 ppm CGA-265307 2.9% TRR <0.001 ppm Unresolved 2.8% TRR <0.001 ppm
Aqueous	65.2	0.007	Not further analyzed.

(continued; footnotes follow)

Fraction	% TRR [a]	ppm	Characterization/Identification
Residual solids	15.8	0.002	

(*continued; footnotes follow*)

Table 40. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
29-day PBI, Wheat forage (0.067 ppm)			
MeOH:H_2O	74.6	0.050	2D-TLC analysis: Thiamethoxam 5.5% TRR 0.004 ppm CGA-322704 7.6% TRR 0.005 ppm NOA-407475 6.3% TRR 0.004 ppm CGA-382191 7.4% TRR 0.005 ppm NOA-421275 13.1% TRR 0.009 ppm CGA-265307 3.3% TRR 0.002 ppm NOA-405217 5.0% TRR 0.003 ppm Desmethyl-CGA-353968 1.9% TRR 0.001 ppm Unknown(s)+ Unresolved 24.3% TRR 0.016 ppm each at ≤6.6% TRR 0.004 ppm
MeOH Soxhlet	11.5	0.008	Not further analyzed.
Residual solids	23.5	0.016	
104-day PBI, Wheat forage (0.056 ppm)			
MeOH:H_2O	81.4	0.046	Concentrated and partitioned between DCM and H_2O.
DCM	19.0	0.011	2D-TLC analysis: Thiamethoxam 2.1% TRR 0.001 ppm CGA-322704 11.4% TRR 0.006 ppm NOA-407475 0.6% TRR <0.001 ppm NOA-421275 0.5% TRR <0.001 ppm CGA-265307 2.5% TRR 0.001 ppm NOA-405217 0.5% TRR <0.001 ppm Unknown+Unresolved 1.5% TRR <0.001 ppm
Aqueous	61.2	0.034	2D-TLC analysis: CGA-322704 2.1% TRR 0.001 ppm NOA-407475 4.9% TRR 0.003 ppm CGA-382191 6.7% TRR 0.004 ppm NOA-421275 8.5% TRR 0.005 ppm CGA-265307 1.2% TRR <0.001 ppm NOA-405217 4.0% TRR 0.002 ppm Desmethyl-CGA-353968 1.7% TRR <0.001 ppm Unknown(s)+ Unresolved 32.1% TRR 0.018 ppm each at ≤7.9% TRR 0.004 ppm
Residual solids	NR	--	Soxhlet extracted with MeOH
MeOH Soxhlet	5.1	0.002	Not further analyzed.
Residual solids	15.9	0.009	
180-day PBI Wheat forage (0.023 ppm)			
MeOH:H_2O	80.9	0.019	Concentrated and partitioned between DCM and H_2O.
DCM	32.8	0.008	2D-TLC analysis: Thiamethoxam 1.6% TRR <0.001 ppm CGA-322704 19.7% TRR 0.005 ppm CGA-265307 2.7% TRR <0.001 ppm Known(s) + Unresolved 8.2% TRR 0.002 ppm
Aqueous	42.2	0.010	Not further analyzed.

(continued; footnotes follow)

Table 40. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
Residual solids	22.3	0.005	
362-day PBI Wheat forage (0.035 ppm)			
MeOH:H_2O	81.3	0.028	Concentrated and partitioned between DCM and H_2O.
DCM	8.4	0.003	2D-TLC analysis: CGA-322704 2.5% TRR <0.001 ppm CGA-265307 3.1% TRR 0.001 ppm Unknown(s)+ Unresolved 2.2% TRR <0.001 ppm
Aqueous	68.0	0.024	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted with MeOH.
MeOH Soxhlet	5.1	0.002	Not further analyzed.
Residual solids	17.0	0.006	
29-day PBI Wheat straw (0.520 ppm)			
MeOH:H_2O	63.8	0.332	2D-TLC analysis: Thiamethoxam 1.9% TRR 0.010 ppm CGA-322704 4.4% TRR 0.023 ppm NOA-407475 0.8% TRR 0.004 ppm CGA-353042 1.2% TRR 0.006 ppm CGA-382191 8.3% TRR 0.043 ppm NOA-421275 7.8% TRR 0.041 ppm NOA-405217 4.7% TRR 0.024 ppm CGA-265307 6.7% TRR 0.035 ppm CGA-353968 0.8%TRR 0.004 ppm Desmethyl CGA-353968 2.8% TRR 0.015 ppm Unknowns+Unresolved 24.4% TRR 0.126 ppm each at ≤8.9% TRR 0.044 ppm Identity of isolated TLC fractions were confirmed by reverse phase TLC analysis with reference standards.
Residual solids	NR	--	Soxhlet extracted with MeOH.
MeOH Soxhlet	7.3	0.038	Not further analyzed.
Residual solids	28.1	0.146	ASE extraction with PrOH:H_2O at 170 C and 1450 psi..
PrOH:H_2O extract	14.6	0.076	Concentrated and resolubized in PrOH:H_2O yield fraction with 11.1% TRR (0.058 ppm). 2D-TLC analysis: Thiamethoxam 1.0% TRR 0.005 ppm CGA-322704 3.7% TRR 0.019 ppm CGA-382191 5.4% TRR 0.028 ppm CGA-265307 5.6% TRR 0.029 ppm Desmethyl CGA-353968 1.4% TRR 0.007 ppm Unknowns+Unresolved 22.5% TRR 0.117 ppm each at ≤10.2% TRR 0.053 ppm
Residual solids	NR	--	Hydrolyzed in boiling 2N NaOH
Hydrolysate	8.7	0.045	Acidified to pH 1, concentrated and centrifuged to precipitate lignin.
Precipitate	3.9	0.020	Radioactivity associated with lignin fraction.
Filtrate	3.1	0.016	Not further analyzed.

(continued; footnotes follow)

Table 40. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
Residual solids	2.2	0.011	
104-day PBI Wheat straw (0.233 ppm)			
MeOH:H_2O	56.2	0.131	Concentrated and partitioned between DCM and H_2O.
DCM	14.6	0.034	Purified by C_{18} column 2D-TLC analysis: Thiamethoxam 1.5% TRR 0.003 ppm CGA-322704 4.9% TRR 0.011 ppm NOA-405217 1.2% TRR 0.003 ppm CGA-265307 4.3% TRR 0.010 ppm Unknown+Unresolved 2.2% TRR 0.005 ppm
Aqueous	43.1	0.100	2D-TLC analysis: CGA-322704 0.9% TRR 0.002 ppm NOA-407475 1.2% TRR 0.003 ppm CGA-382191 5.3% TRR 0.012 ppm NOA-421275 4.9% TRR 0.011 ppm NOA-405217 8.7% TRR 0.020 ppm CGA-265307 1.5% TRR 0.003 ppm Desmethyl-CGA-353968 1.4% TRR 0.003 ppm Unknown(s)+ Unresolved 19.1% TRR 0.045 ppm each at ≤4.7% TRR 0.011ppm
Residual solids	NR	--	Soxhlet extracted with MeOH.
MeOH Soxhlet	3.5	0.008	Not further analyzed.
Residual solids	36.4	0.085	ASE extraction with PrOH:H_2O at 170 C and 1450 psi..
PrOH:H_2O extract	16.9	0.039	2D-TLC analysis: CGA-322704 1.7% TRR 0.004 ppm NOA-405217 1.7% TRR 0.004 ppm CGA-265307/Desmethyl CGA-353968 2.9% TRR 0.007 ppm Unknown+Unresolved 10.6% TRR 0.025 ppm each at ≤8.4% TRR 0.020 ppm
Residual solids	17.3	0.040	Not further analyzed.
180-day PBI Wheat straw (0.057 ppm)			
MeOH:H_2O	65.6	0.037	Concentrated and partitioned between DCM and H_2O.
DCM	10.0	0.006	Cleaned up on a C18 column eluted with H_2O and MeOH.
Aqueous	1.5	<0.001	Not further analyzed.
MeOH	8.9	0.005	2D-TLC analysis: Thiamethoxam 0.3% TRR <0.001 ppm CGA-322704 3.2% TRR 0.002 ppm CGA-265307 3.2% TRR 0.002 ppm Unknown + Unresolved 2.1% TRR 0.001 ppm
Aqueous	51.6	0.029	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted in MeOH.
MeOH Soxhlet	4.3	0.002	Not further analyzed.

(continued; footnotes follow)

Table 40. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
Residual solids	36.9	0.021	
362-day PBI Wheat straw (0.080 ppm)			
MeOH:H_2O	56.1	0.045	Concentrated and partitioned between DCM and H_2O.
DCM	10.3	0.008	Cleaned up on a C18 column eluted with H_2O and MeOH.
Aqueous	1.8	0.001	Not further analyzed.
MeOH	8.1	0.006	2D-TLC analysis: CGA-322704 2.8% TRR 0.002 ppm CGA-265307 3.3% TRR 0.002 ppm Unknown + Unresolved 2.1% TRR 0.002 ppm
Aqueous	42.5	0.034	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted in MeOH.
MeOH Soxhlet	3.6	0.003	Not further analyzed.
Residual solids	40.4	0.032	
29-day Wheat grain (0.020 ppm)			
MeOH:H_2O	50.9	0.010	Concentrated and partitioned between DCM and H_2O.
DCM	13.0	0.003	2D-TLC analysis: Thiamethoxam 1.0% TRR <0.001 ppm CGA-322704 3.3% TRR <0.001 ppm CGA-265307 7.3% TRR 0.001 ppm Unresolved 1.3% TRR <0.001 ppm
Aqueous	37.4	0.007	2D-TLC analysis: 8 unknowns 37.4% TRR 0.007 ppm each at ≤6% TRR 0.001 ppm .
Residual solids	NR	--	Soxhlet extracted in MeOH.
MeOH Soxhlet	8.5	0.002	Not further analyzed.
Residual solids	41.1	0.008	
104-day PBI Wheat grain (0.085 ppm)			
MeOH:H_2O	17.2	0.014	Concentrated and partitioned between DCM and H_2O.
DCM	3.8	0.003	Cleaned up on a C_{18} column (88.4% recovery) 2D-TLC analysis: CGA-322704 0.3% TRR <0.001 ppm CGA-265307 1.4% TRR 0.001 ppm Unknowns + Unresolved 1.9% TRR 0.002 ppm each at ≤0.9% TRR <0.001 ppm
Aqueous	11.6	0.010	2D-TLC analysis: CGA-265307 0.9% TRR <0.001 ppm Desmethyl CGA-353968 0.5% TRR <0.001 ppm Unknowns + Unresolved 10.3% TRR 0.009 ppm each at ≤7.1% TRR ≤0.006 ppm
Precipitate	1.7	0.001	Not further analyzed.
Residual solids	NR	--	Soxhlet extracted in MeOH.

(continued; footnotes follow)

Table 40. Continued.

Fraction	% TRR [a]	ppm	Characterization/Identification
MeOH Soxhlet	2.5	0.002	Not further analyzed.
Residual solids	82.3	0.070	Extracted with 0.05N NaOH, neutralized, and proteins precipitated with EtOH.
Extract	7.7	0.007	Not further analyzed.
Precipitate	9.6	0.008	Radioactivity associated with protein fraction.
Residual solids	NR	--	Hydrolyzed by refluxing overnight in 1 N HCl
Acid hydrolysate	57.2	0.048	Neutralized and partitioned with DCM
DCM	0.9	<0.001	Not further analyzed.
Aqueous	56.8	0.048	Derivatized, recrystallized, and filtered to yield glucosazone and filtrate fractions. An additional aliquot was further fractionated and purified by anion and cation exchange chromatography; however, no fractions suitable for TLC analysis were obtained.
Starch	26.7	0.023	Identified as being incorporated into starch.
Filtrate	30.0	0.026	Not further analyzed
Residual solids	4.7	0.004	Not further analyzed.

[a] Not corrected for recovery.

Characterization and identification of residues

^{14}C-Residues in solvent extracts and in DCM and aqueous fractions were analyzed and quantified by 2D-TLC using silica gel plates with tetrahydrofuran:MeOH:formic acid:H_2O (60:35:1:4) and chloroform:MeOH:formic acid:H_2O (75:20:4:2). ^{14}C-Residues were detected using a radioanalytic imaging system and quantified by LSC. With two exceptions, reference compounds were detected using UV light; CGA-353042 and NOA-405217 were visualized using a reagent specific for visualizing guanidines. A total of 13 reference standards, including parent, were used for comparison. ^{14}C-Residues were identified by co-chromatography with reference standards. For confirmation of metabolite identities, metabolites were isolated by TLC from 29-day PBI samples of lettuce, radish tops, and wheat straw and were co-chromatographed with reference standards using reverse-phase TLC analysis. The characterization and identification of ^{14}C-residues in rotational crops grown in soil treated with [^{14}C]thiamethoxam at ~200 g ai/ha (~0.8x) are summarized in Tables 41 and 42. Residues in wheat matrices from the 180-day PBI are not included in the summary tables as the residue profile in these samples was similar to wheat samples from the 104-day PBI.

The metabolic profile of ^{14}C-residues in rotational crops was qualitatively and quantitatively similar for the two ^{14}C-labels, with the exception of the [oxadiazin-^{14}C]label specific metabolites, CGA-353042, NOA-405217, and CGA-382191.

In lettuce, thiamethoxam and CGA-322704 were the principal metabolites identified from both the 29- and 119-day PBIs. For both ^{14}C-labels, thiamethoxam accounted for 12-25% TRR and CGA-322704 accounted for 12-16% TRR; however, levels of both compounds were <0.01 ppm at both PBIs. In addition, minor amounts of NOA-407475 (5% TRR) and NOA-421275 (5-8% TRR) were detected in samples of both ^{14}C-labels from the 29-day PBI. In [oxadiazin-^{14}C]-treated lettuce, minor amounts of the NOA-405217 (11% TRR) and CGA-382191 (5% TRR) were also detected. Each of these metabolites was present at <0.01 ppm.

^{14}C-Residues in radish roots were too low (<0.01 ppm) for analysis; however, thiamethoxam and up to 10 metabolites were identified in radish tops. In radish tops from the 29-day PBI, thiamethoxam accounted for 20-24% TRR (0.019-0.023 ppm). Metabolites present at >0.01 ppm included CGA-322704 at 7-16% TRR (0.008-0.012 ppm) and CGA-265307 at 9% TRR (0.007-0.011 ppm). For both ^{14}C-labels, minor amounts of NOA-407475 (7-9% TRR), NOA-421275 (6-8% TRR), desmethyl-CGA-353968 (2% TRR), and CGA-355190 (1% TRR) were also detected, with each present at <0.01 ppm. Minor amounts of CGA-353968 (5% TRR) were also detected only in the [thiazol-^{14}C]-treated tops, and NOA-405217 and CGA-382191 (3-6% TRR) were detected only in [oxadiazin-^{14}C]-treated tops. By the 119-day PBI, residues in [thiazol-^{14}C]-treated radish tops were too low for analysis, and identified metabolites in [oxadiazin-^{14}C]-treated plants were all ≤0.001 ppm.

In wheat forage, the only metabolites present at >0.01 ppm were CGA-322704 (10% TRR, 0.011 ppm) and NOA-421275 (21% TRR, 0.023 ppm) in [thiazol^{14}C]-treated forage from the 29-day PBI. Although numerous other metabolites and parent were identified in forage, none of these were present at ≥0.01 ppm. At the 29-day PBI, the principal residues in forage consisted of CGA-322704 (8-10% TRR) and NOA-421275 (13-21%TRR) for both ^{14}C-labels. Parent accounted for 4-6% TRR, and other minor metabolites included: NOA-407475 (6% TRR), CGA-265307 (3-4% TRR), desmethyl-CGA-353968 (1-2% TRR), NOA-405217 (5% TRR) and CGA-382191 (7% TRR). The metabolic profile was the same in forage from the 104-day PBI, with CGA-322704 (14-19% TRR) and NOA-421275 (9% TRR) again being the principal residues identified. Parent and the other metabolites each accounted for 2-7% TRR. By the 362-day PBI, only CGA-322704 and CGA-265307 were detected in wheat forage from both ^{14}C-labels at 3-4% TRR (≤0.001 ppm).

The metabolic profile in wheat straw was similar to the profile in forage, except that numerous metabolites were present at levels >0.01 ppm as TRR levels in straw were considerably higher than in forage. At the 29-day PBI, parent accounted for 3-5% TRR (0.015-0.038 ppm) and CGA-322704 accounted for 7-8% TRR (0.042-0.052 ppm). Other metabolites which contributed substantially to the residue included: NOA-421275 (8-9% TRR, 0.041-0.066), CGA-265307 (6-12% TRR, 0.047-0.064 ppm) and CGA-382191 (14% TRR, 0.071 ppm). In addition, NOA-

407475 (1-3% TRR), desmethyl-CGA-353968 (3-4% TRR), and NOA-405217 (5% TRR) were each present at 0.022-0.025 ppm. The metabolites CGA-353042 and CGA-353968 were also detected in 29-day PBI wheat straw, but were at <0.01 ppm. By the 104-day PBI, metabolites present at >0.01 ppm included: CGA-322704 (7-8% TRR, 0.011-0.017 ppm), CGA-265307 (9-10% TRR, 0.017-0.020 ppm), NOA-405217 (12% TRR, 0.027 ppm) and CGA-382191 (5% TRR, 0.012 ppm). Parent and desmethyl-CGA-353968 were both detected in straw from the 104-day PBI at 1-4% TRR, but each accounted for <0.01 ppm. No specific residues were present at ≥0.01 ppm in straw by the 180- or 362-day PBI. As with forage, the primary metabolites CGA-322704 and CGA-265307 (3-5% TRR, ≤0.004 ppm) were the only residues detected in straw from the 362-day PBI.

In wheat grain, no specific residues were present at ≥0.01 ppm from either ^{14}C-label at any PBI. At the 29-day PBI, thiamethoxam (0.3-1% TRR), CGA-322704 (3-4% TRR) and CGA-265307 (7% TRR) were the only residues identified in grain. At the 104-day PBI, parent was not detected, but CGA-322704 and CGA-265307 were still detected at ≤2% TRR, along with desmethyl-CGA-353968 at 0.5% TRR. In addition, 17-27% of the TRR in grain from the 104-day PBI was shown to have been incorporated into starch/glucose. Residues in grain from the 180- and 362-day PBIs were too low for analysis.

Along with the data on ^{14}C-residues in rotational crops, the petitioner also presented some data on ^{14}C-residues in the treated soil over time. Immediately following application, thiamethoxam was the only ^{14}C-residue detected in extracts of soil. At subsequent sampling intervals, residues of thiamethoxam *per se* declined steadily in the soil both in absolute and relative (%TRR) levels. The major metabolite identified in soil was CGA-322704, which accounted for up to 30-36% of the TRR in the soil at the later PBIs. Other minor metabolites detected in soil included: NOA-405217 (≤9.1% TRR), CGA-265307 (≤4.8% TRR), and CGA-355190 (≤4.1% TRR).

Summary

Although this study was also not conducted at 1x the maximum seasonal rate, the study is acceptable as ^{14}C-residues were sufficiently identified/characterized and the available data allow HED to conclude that limited field rotational crop studies are necessary to support the proposed 120-day PBI.

Following a soil application of [^{14}C]thiamethoxam at ~200 g ai/ha (~0.8x), radioactive residues were generally low (≤0.05 ppm) in RACs, with the exceptions of wheat straw from each PBI, and wheat forage and radish tops from the 29-day PBI, and wheat grain from the 104-day PBI. As in the other confined rotational crop study, ^{14}C-residues were lowest in root matrices (≤0.007 ppm) and highest in straw (0.051-0.753 ppm). With the exception of the anomalous results for wheat grain from the 104-day PBI, ^{14}C-residues were greatest at the 29-day PBI and decreased at subsequent PBIs. Total radioactive residues were <0.01 ppm in lettuce, radishes, and wheat grain At the 362-day PBI.

Radioactive residues in rotational crops were adequately identified or characterized. With the exception of several metabolites specific to the [oxadiazin-^{14}C]-label, the metabolic profiles for the two ^{14}C-labels were similar qualitatively and quantitatively for each matrix.

The principal residues identified in lettuce and radish tops at the earliest PBI (29 days) included parent (20-25% TRR) and CGA-322704 (7-16% TRR), although substantial portions of the ^{14}C-residue were also accounted for by the following metabolites: CGA-265307 (9% TRR), CGA-353968 (5% TRR), NOA-407475 (5-9% TRR), NOA-421275 (5-8% TRR), NOA-405217 (3-11% TRR), and CGA-382191 (5-6% TRR). However, components present at ≥0.01 ppm were found only in radish tops and included: thiamethoxam (0.019-0.023 ppm), CGA-322704 (0.012 ppm), CGA-265307 (0.011 ppm). All residues were present at <0.01 ppm in lettuce from the 29-day PBI. At the 119-day PBI, only thiamethoxam, CGA-322704, and CGA-265307 were detected in lettuce and radish tops, and were each present at <0.01 ppm.

The principal ^{14}C-residues identified in wheat forage from the 29-, 104-, and 180-day PBIs, were CGA-322704 (8-31% TRR, 0.004-0.011 ppm) and NOA-421275 (9-21% TRR, 0.003-0.023 ppm), and these compounds were present at ≥0.01 ppm only in forage from the 29-day PBI. The other components identified in forage each accounted 1-7% of the TRR (<0.01 ppm) and included: thiamethoxam, CGA-265307, desmethyl-CGA-3523968, NOA-407475, NOA-405217, and CGA-382191.

The metabolic profile in wheat straw was qualitatively similar to the profile in forage; however, no individual metabolite comprised a major portion of the TRR and numerous metabolites were present at levels >0.01 ppm. At the 29-day PBI, thiamethoxam accounted for 3-5% TRR and 0.015-0.038 ppm. Metabolites detected at ≥0.01 and accounting for 3-12% TRR included: CGA-322704 (0.042-0.052 ppm), CGA-265307 (0.047-0.064 ppm), NOA-421275 (0.041-0.066), CGA-382191 (0.071 ppm), NOA-407475 (0.023 ppm), desmethyl-CGA-353968 (0.022-0.025 ppm), and NOA-405217 (0.024 ppm). By the 104-day PBI, metabolites present at 0.013-0.027 ppm included: CGA-322704, CGA-265307, NOA-407475, NOA-405217, and CGA-382191. No specific metabolites were present at ≥0.01 ppm in straw by the 362-day PBI.

The only residues detected in wheat grain from any PBI were thiamethoxam (≤1% TRR), CGA-322704 (≤4% TRR) and CGA-265307 (7% TRR), each of which were present at ≤0.002 ppm.

Conclusions

Although neither study was conducted at 1x the maximum seasonal rate (0.215 lb ai/A/season based on cotton) for rotated crops, these confined rotational crop studies are acceptable as ^{14}C-residues were sufficiently identified/characterized and the available data allow HED to conclude that limited field rotational crop studies are necessary to support the proposed 120-day PBI for rotational crops. In addition, these studies indicate that the metabolism of [^{14}C]thiamethoxam in rotational crops is similar to the metabolism observed in primary crops (corn, cucumbers, and pears).

Results from the two studies were comparable. As expected, ^{14}C-residues resulting from the 200 g ai/ha application were initially higher than residues resulting from the 100 g ai/ha application; however, the relative distribution of ^{14}C-residues among the various RACs was the same in each study. In addition, the metabolic profile for the two studies was similar, although more metabolites were identified in the second study due to the higher levels of ^{14}C-residues.

In a meeting held on 7/28/99 (see memo of G.J. Herndon dated 8/31/99), the Metabolism Assessment Review Committee (MARC) determined that the major residues from the confined rotational crop studies were the parent thiamethoxam and its CGA-322704 metabolite. CGA-265307 was a major residue still having the N-nitro group in animal feed items (e.g. wheat straw). All three compounds should be analyzed in field rotational crop studies.

The proposed label for Helix specifies the following rotational crop restrictions: treated areas may be replanted immediately following harvest or as soon as practical following the last application with wheat or canola. Barley, cole crops, cotton, cucurbit vegetables, fruiting vegetables, leafy vegetables, pome fruit, sorghum, tobacco, tuberous and corm vegetables may be planted 30 days after the last application of Helix. For all other crops, a 120-day plantback interval must be observed.

The proposed use of Helix as a seed treatment on canola specifies that a maximum rate of 0.4 lb.ai./100 lbs. of seed. Using a typical seeding rate for canola of 10 lbs./A. (source: Bernie Schneider, HED), this equates to 0.04 lbs.ai./A.. The confined rotational crop study for which the petitioner performed identification work was conducted at about 0.09 lb.ai./A. to the soil. Therefore, for the proposed use of thiamethoxam as a seed treatment use of canola, the confined rotational crop study was conducted at about a 2.25X rate. At a 30-day plantback interval, residues of thiamethoxam and CGA-322704 were a maximum of 0.006 ppm in mustard leaves, 0.026 ppm in turnip tops, 0.015 ppm in wheat forage, 0.02 ppm in wheat straw, and 0.003 ppm in wheat grain. The LOQ of the proposed enforcement method is 0.02 ppm (0.01 ppm for each thiamethoxam and CGA-322704). Therefore, based on the exaggerated rate (2.25X) and low residues found, **RAB2 believes that, for the proposed use of thiamethoxam as a seed treatment use on canola, a 30-day plantback interval is appropriate for all crops (except canola, which may be replanted at any time).**

Table 41. Summary of the characterization/identification of radioactive residues in RACs from rotational crops following an application of [**thiazol**-^{14}C]thiamethoxam to the soil at ~200 g ai/ha (~0.8x maximum seasonal rate).

Metabolite/ fraction	Lettuce leaves		Radish tops		Wheat forage		Wheat straw		Wheat grain	
	%TRR [a]	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
29-day Plant-back Interval										
Thiamethoxam	21.3	0.008	19.8	0.023	4.4	0.005	5.1	0.038	0.3	<0.001
CGA-322704	12.1	0.004	6.8	0.008	10.0	0.011	6.9	0.052	3.8	0.001
CGA-265307	-- [c]	--	9.1	0.011	3.7	0.004	6.3	0.047	7.4	0.002
CGA-355190	--	--	0.9	0.001	--	--	--	--	--	--
CGA-353968	--	--	5.2	0.006	--	--	--	--	--	--
Desmethyl-CGA-353968	--	--	2.0	0.002	1.2	0.001	3.4	0.025	--	--
NOA-407475	4.5	0.002	6.7	0.008	6.3	0.007	3.0	0.023	--	--
NOA-421275	8.2	0.003	6.3	0.007	20.7	0.023	8.7	0.066	--	--
Total identified	**46.1**	**0.017**	**56.8**	**0.066**	**46.3**	**0.05**	**33.4**	**0.251**	**11.5**	**0.003**
TLC unknown(s) [d]	21.2	0.008	16.2	0.019	22.6	0.025	25.0	0.188	0.3	<0.001
Unresolved ^{14}C-residue [e]	10.8	0.004	7.1	0.008	8.9	0.010	9.3	0.070	1.8	<0.001
Aqueous fraction(s)	--	--	--	--	--	--	4.9	0.036	31.7	0.009
Organic fraction(s)	1.5	<0.001	1.6	0.002	5.6	0.006	3.3	0.025	3.1	<0.001
Lignin fraction	--	--	--	--	--	--	2.1	0.016		
Total identified/ characterized	**79.6**	**0.030**	**81.7**	**0.095**	**83.4**	**0.09**	**78.0**	**0.586**	**48.4**	**0.014**
Residual solids	17.3	0.006	9.4	0.0011	9.4	0.011	1.6	0.012	65.1	0.019
104/119-day Plant-back Interval [f]										
Thiamethoxam	18.4	0.002	--	--	--	--	4.1	0.009	--	--
CGA-322704	15.9	0.002	--	--	18.8	0.006	7.7	0.013	0.2	<0.001
CGA-265307	--	--	--	--	4.7	0.001	9.9 [g]	0.017	1.2 [g]	0.002
Desmethyl-CG 353968	--	--	--	--	2.0	<0.001	1.6	0.003	--	--
NOA-407475	--	--	--	--	1.6	<0.001	--	--	--	--
NOA-421275	--	--	--	--	9.3	0.003	4.8	0.008	--	--
Total identified	**34.3**	**0.004**	--	--	**36.4**	**0.011**	**28.1**	**0.050**	**1.4**	**0.002**
TLC unknown(s) [d]	--	--	--	--	25.3	0.008	22.1	0.038	4.1	0.006
Unresolved ^{14}C-residue [e]	8.1	0.001	--	--	19.4	0.006	16.0	0.027	3.7	0.005
Aqueous fraction(s)	33.6	0.004	81.5	0.009	--	--	--	--	46.1	0.068
Organic fraction(s)	--	--			6.8	0.002	6.9	0.012	2.3	0.003
Starch [h]	--	--	--	--	--	--	--	--	16.8	0.025
Protein fraction [i]	--	--	--	--	--	--	--	--	8.8	0.013
Total identified/ characterized	**76.0**	**0.010**	**81.5**	**0.009**	**87.9**	**0.03**	**73.1**	**0.127**	**83.2**	**0.122**
Residual solids	24.9	0.003	23.1	0.003	19.6	0.006	21.4	0.037	12.0	0.017

(continued; footnotes follow)

Table 41. Continued.

Metabolite/ fraction	Lettuce leaves		Radish tops		Wheat forage		Wheat straw		Wheat grain	
	%TRR [a]	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
362-day Plant-back Interval										
CGA-322704	not analyzed		not analyzed		4.3	<0.001	5.4	0.004	not analyzed	
CGA-265307					3.8	<0.001	4.3	0.004		
Total identified					**8.1**	**0.002**	**9.7**	**0.008**		
TLC unknown(s) [d]					1.1	<0.001	0.8	<0.001		
Unresolved ^{14}C-residue [e]					2.7	<0.001	2.0	0.002		
Aqueous fraction(s)					64.3	0.012	39.3	0.032		
Organic fraction(s)					5.3	0.001	3.2	0.002		
Total identified/ characterized					**81.5**	**0.015**	**55.0**	**0.045**		
Residual solids					24.5	0.005	44.1	0.036		

[a] %TRR not corrected for recovery.
[b] Expressed in [^{14}C]thiamethoxam equivalents.
[c] -- = Not detected.
[d] Specific unknowns isolated by TLC each accounted for ≤10.8% of the TRR.
[e] Radioactivity on TLC plates not associated with a specific region.
[f] Lettuce and radish data are from a 119-day PBI and wheat data are from 104-day PBI.
[g] Fraction includes minor amounts (<1% TRR) of desmethyl-CGA-353968.
[h] Identified as being incorporated into starch.
[i] Radioactivity in associated with the isolated protein fraction.

Table 42. Summary of the characterization/identification of radioactive residues in RACs from rotational crops following an application of [**oxadiazin-**^{14}C]thiamethoxam to the soil at ~200 g ai/ha (~0.8x maximum seasonal rate).

Metabolite/ fraction	Lettuce leaves		Radish tops		Wheat forage		Wheat straw		Wheat grain	
	%TRR [a]	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
29-day Plant-back Interval										
Thiamethoxam	25.1	0.009	24.4	0.019	5.5	0.004	2.9	0.015	1.0	<0.001
CGA-322704	11.9	0.004	15.5	0.012	7.6	0.005	8.1	0.042	3.3	<0.001
CGA-265307	-- [c]	--	8.9	0.007	3.3	0.002	12.3	0.064	7.3	0.001
CGA-355190	--	--	1.3	0.001	--	--	--	--	--	--
CGA-353968	--	--	--	--	--	--	0.8	0.004	--	--
Desmethyl-CGA-353968	--	--	1.8	0.001	1.9	0.001	4.2	0.022	--	--
NOA-407475	5.0	0.002	9.2	0.007	6.3	0.004	0.8	0.004	--	--
NOA-421275	5.4	0.002	7.5	0.006	13.1	0.009	7.8	0.041	--	--
CGA-353042	--	--	--	--	--	--	1.2	0.006	--	--
NOA-405217	11.2	0.004	2.8	0.002	5.0	0.003	4.7	0.024	--	--
CGA-382191	4.8	0.002	6.4	0.005	7.4	0.005	13.7	0.071	--	--
Total identified	**63.4**	**0.023**	**77.8**	**0.060**	**50.1**	**0.030**	**56.5**	**0.293**	**11.6**	**0.002**
TLC unknown(s) [d]	8.5	0.003	6.7	0.005	17.7	0.012	37.1	0.193	37.4	0.007
Unresolved ^{14}C-residue [e]	20.5	0.007	10.4	0.008	6.6	0.004	9.8	0.051	1.3	<0.001
Aqueous fraction(s)	--	--	--	--	--	--	3.1	0.016	--	--
Organic fraction(s)	1.9	<0.001	2.2	0.002	11.5	0.008	7.3	0.038	8.5	0.002
Lignin fraction	--	--	--	--	--	--	3.9	0.020	--	--
Total identified/ characterized	**94.3**	**0.034**	**97.1**	**0.075**	**85.9**	**0.050**	**117.7**	**0.611**	**58.8**	**0.012**
Residual solids	14.5	0.005	7.8	0.006	23.5	0.016	2.2	0.011	41.1	0.008

(continued; footnotes follow)

Table 42. Continued.

Metabolite/ fraction	Lettuce leaves		Radish tops		Wheat forage		Wheat straw		Wheat grain	
	%TRR [a]	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
104/119-day Plant-back Interval [f]										
Thiamethoxam	12.2	0.002	9.7	0.001	2.1	0.001	1.5	0.003	--	--
CGA-322704	13.7	0.002	6.5	<0.001	13.5	0.007	7.5	0.017	0.3	<0.001
CGA-265307	--	--	2.9	<0.001	3.7	0.002	8.7 [g]	0.020	2.3	0.002
Desmethyl-CGA-353968	--	--	--	--	1.7	<0.001	1.4	0.003	0.5	<0.001
NOA-407475	--	--	--	--	5.5	0.003	1.2	0.003	--	--
NOA-421275	--	--	--	--	9.0	0.005	4.9	0.011	--	--
NOA-405217	--	--	--	--	4.5	0.003	11.6	0.027	--	--
CGA-382191	--	--	--	--	6.7	0.004	5.3	0.012	--	--
Total identified	**25.9**	**0.004**	**19.1**	**0.002**	**46.7**	**0.026**	**42.1**	**0.096**	**3.1**	**0.003**
TLC unknown(s) [d]	2.6	<0.001	--	--	20.5	0.011	15.1	0.035	4.7	0.004
Unresolved ^{14}C-residue [e]	5.8	<0.001	2.8	<0.001	13.1	0.007	16.8	0.040	7.5	0.006
Aqueous fraction(s)	48.4	0.006	65.2	0.003	--	--	--	--	37.7	0.032
Organic fraction(s)	--	--	--		5.1	0.002	3.5	0.008	3.4	0.003
Starch [h]	--	--	--	--	--	--	--	--	26.7	0.023
Protein fraction [i]	--	--	--	--	--	--	--	--	9.6	0.008
Total identified/ characterized	**82.7**	**0.010**	**87.1**	**0.010**	**85.4**	**0.048**	**77.5**	**0.188**	**92.7**	**0.079**
Residual solids	19.2	0.002	15.8	0.002	15.9	0.009	17.3	0.040	4.7	0.004
362-day Plant-back Interval										
CGA-322704	not analyzed		not analyzed		2.5	<0.001	2.8	0.002	not analyzed	
CGA-265307					3.1	0.001	3.3	0.002		
Total identified					**5.6**	**0.002**	**6.1**	**0.004**		
TLC unknown(s) [d]					0.7	<0.001	0.5	<0.001		
Unresolved ^{14}C-residue [e]					1.5	<0.001	1.6	0.001		
Aqueous fraction(s)					68.0	0.024	44.3	0.035		
Organic fraction(s)					5.1	0.002	3.6	0.003		
Total identified/ characterized					**80.9**	**0.028**	**56.1**	**0.045**		
Residual solids					17.0	0.006	40.4	0.032		

[a] Expressed in [^{14}C]thiamethoxam equivalents.
[b] Results from the analysis of husks were similar results from wheat straw.
[c] -- = Not detected.
[d] Specific unknowns isolated by TLC each accounted for ≤7.9% of the TRR.
[e] Radioactivity on TLC plates not associated with a specific region.
[f] Lettuce and radish data are from a 119-day PBI and wheat data are from 104-day PBI.
[g] Fraction includes minor amounts (<1% TRR) of desmethyl-CGA-353968.
[h] Identified as being incorporated into starch.

Table 42. Continued.

Radioactivity associated with the isolated protein fraction.

OPPTS GLN 860.1900: Field Accumulation in Rotational Crops

Data from a limited field trial study (1998; MRID 44715106) are under concurrent review by the Agency in conjunction with a separate petition (PP#9F5046) for tolerances of thiamethoxam on various crop commodities.

In a meeting held on 7/28/99 (see memo of G.J. Herndon dated 8/31/99), the Metabolism Assessment Review Committee (MARC) determined that if a field rotational crop study is needed, the parent thiamethoxam plus the CGA-322704 and CGA-265307 metabolites should be analyzed.

CODEX ISSUES

As there are no established Codex MRLs established for residues of thiamethoxam in/on canola, a discussion of compatibility with U.S. tolerances is not relevant at this time.

cc: PP#9F5046, PP#9F5046, RAB2 Reading File, G.J. Herndon