1

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Atrazine (080803), Reregistration Case No. 0062 and Special Review. Field Metabolism Study in Sugarcane (MRID 43016503), Analytical Methods (MRID 43016504), and Field Metabolism Study in Rotational Crops (MRID 43016505).
CBRS No. 12889, DPBarcode No. D197234.

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In response to previous reviews and a reregistration Data Call-In of 10/90, Ciba-Geigy Corporation submitted data on the nature of the residue in sugarcane, analytical method, and nature of the residue in field rotational crops. Assignment instructions are to review the submission. CBRS has previously reported to SRRD residue data on sugarcane as provided by registrant in this submission (Memo, 1/26/94, J. Abbotts). The present memo represents a review of this submission. Conclusions and Recommendations below pertain only to data in the present submission.

Tolerances are established for residues of the herbicide atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, in or on agricultural commodities (40 CFR 180.220(a)), and for combined residues of atrazine and its metabolites 2-amino-4-chloro-6-ethylamino-*s*-triazine, 2-amino-4-chloro-6-isopropylamino-*s*-triazine, and 2-chloro-4,6-diamino-*s*-triazine, in or on specified plant commodities (40 CFR 180.220(b)). Atrazine is a List A Chemical. The Residue Chemistry Chapter was issued 7/25/83; the Registration Standard (Guidance Document) was issued 9/85; a Second Round Review (SRR) Residue Chemistry Chapter was issued 10/18/88. Special Review has been initiated on triazine herbicides, including atrazine (59 FR 60412, 11/23/94).

Conclusions

1. Conclusions under this heading apply to the sugarcane field metabolism study:

1a. The application pattern represented the maximum rate per crop, the maximum rate at planting, the maximum number of postemergence applications, and included an application during the last stage allowed by registered use. The combination of applications chosen was appropriate for a metabolism study, and should produce a range of both early and later metabolites.

1b. The sugarcane field metabolism study was conducted at one site in CA. Geographic variability of residues is not known.

1c. Atrazine undergoes extensive metabolism in sugarcane. The Registrant has identified chloro, hydroxy, amino, and conjugated metabolites, each containing an intact triazine ring (for details, see Table 5 and Figures 4 through 6 of this review). Using Extraction Method I, residues identified represented 26.9% TRR in final harvest leaves, and 52.5% TRR in final harvest cane. Atrazine and other chloro compounds combined represented 5% TRR in final harvest cane, and less than 2% TRR in final harvest leaves.

1d. With Extraction Method II, an acid autoclave procedure, approximately two-thirds of the TRR in final harvest leaves and cane was converted to cyanuric acid and the atrazine hydroxy metabolites G-34048, GS-17794, and GS-17792. This observation indicates that the position of the HED Metabolism Committee, that TRR should represent total residues containing the triazine ring, is a reasonable assumption.

1e. In the present submission, combined residues of parent and chloro metabolites in cane were 0.108 ppm. CBRS previously determined combined anticipated residues of parent and chloro metabolites of 0.13 ppm based on field trial data (DEB 3688-3703, 9/14/88, M.S. Metzger). This value for anticipated residues

should still be considered valid, because the present submission was a metabolism study in a single location. However, in accordance with the present position of the HED Metabolism Committee that exposure assessment should be conducted on the basis of TRR, 2.52 ppm should be used for sugarcane cane for total residues containing the triazine ring, based on data in the present submission. SRRD has previously been advised of the residue data for sugarcane reported in the present submission (Memo, 1/26/94, J. Abbotts).

1f. Further data on field metabolism in sugarcane are not required.

Conclusion 2. The conclusions under this heading apply to the rotational crop field metabolism studies:

2a. Atrazine undergoes extensive metabolism in the rotational crops wheat, spinach, and garden beets. The Registrant has identified chloro, hydroxy, amino, and conjugated metabolites, each containing an intact triazine ring (for details, see Tables 11 and 12 and Figures 4 through 6 of this review). Using Extraction Method I, residues identified were as low as 4.7% TRR in a mature grain sample, and as high as 76.8% TRR in a mature spinach sample. The distribution of residues varied with crop, with commodity of the same crop, and with geographical location. Atrazine and other chloro compounds combined represented less than 2% TRR in samples of mature grain and mature beet tops, and up to 74% TRR in a sample of mature spinach leaves.

2b. When Extraction Method II, an acid autoclave procedure, was applied to samples from IL, from 52% TRR to 86% TRR in mature rotational crops was converted to cyanuric acid and the atrazine hydroxy metabolites G-34048, GS-17794, and GS-17792. These observations indicate that the position of the HED Metabolism Committee, that TRR should represent total residues containing the triazine ring, is a reasonable assumption for rotational crops.

2c. Metabolic pathways are similar for rotational crops and primary crops, at least for early metabolites. No further data are required for field metabolism studies of rotational crops.

2d. The field metabolism studies are sufficient to meet the requirements for limited field rotational crop studies.

3. The present submission is considered relevant to the early metabolic pathway of atrazine from wheat fallow application, but is inadequate to determine the magnitude of total triazine ring residues resulting from such use.

Conclusion 4: Conclusions under this heading apply to analytical methods.

4a. Recoveries from fortified samples were adequate for Methods AG-484 and AG-596. The limits of determination for Method AG-484 were the lowest levels analyzed, 0.05 ppm in sugarcane leaves and cane and in wheat forage and straw, and 0.01 ppm in wheat grain, for each of parent atrazine and chloro metabolites G-30033, G-28279, and G-28273. The limits of determination for Method AG-596 were the lowest levels analyzed, 0.025 ppm in wheat grain and 0.02 ppm in sugarcane leaves and cane and in wheat forage and straw, for each of the hydroxy metabolites G-34048 and GS-17794.

4b. Radiovalidation data for Method AG-484 were sufficient to validate the ability of the method to detect residues of parent atrazine and G-30033 near the limits of determination in wheat forage, and residues of G-28273 near 0.4 ppm. For G-28279, radiovalidation confirmed the limits of determination in sugarcane and wheat samples.

4c. Radiovalidation data for Method AG-596 were sufficient to validate the ability of the method to detect residues of GS-17794 at levels of 0.12 ppm or higher. This method is not considered radiovalidated for compound G-34048.

Recommendations

In accordance with Conclusions 2c and 2d above, the present submission satisfies reregistration data requirements for Guideline 165-1, Confined Rotational Crops, and Guideline 165-2, Field Rotational Crops, Limited Trials. Depending on the tolerance expression and the limits of quantitation of the enforcement method, it is expected that extensive field trials will be required to support reregistration for rotational crops. CBRS advises that a more definitive determination of the tolerance expression, and therefore of requirements for rotational crop field trials, should be available after review of the hydroxyatrazine cancer study. Field trials should be conducted with non-radioactive test substance, but conservative assumptions will be used to estimate total triazine ring residues for exposure assessment. We also advise that dietary exposure to atrazine residues is likely to be increased by residues on foods from rotational crops.

In accordance with Conclusion 3, the requirements of the 10/90 DCI for data on wheat fallow use have not been met. Data are still required on wheat fallow application that would at least indicate a ratio between combined residues of parent and chloro metabolites:total triazine ring residues. These data could best be provided by a radiolabel study that determines TRR and residues of parent and chloro metabolites.

DETAILED CONSIDERATIONS

NATURE OF THE RESIDUE

Reregistration Requirements

The Residue Chemistry Chapter (7/25/83) concluded that the metabolism of atrazine in plants was adequately understood. Identified metabolites included 2-chloro-4,6-diamino-*s*-triazine (G-28273), 2-amino-4-chloro-6-ethylamino-*s*-triazine (G-28279), 2-amino-4-chloro-6-isoproylamino-*s*-triazine (G-30033), and 2-ethylamino-4-isopropylamino-6-hydroxy-*s*-triazine (hydroxyatrazine, G-34048). The Second Round Review Residue Chemistry Chapter (10/18/88) reported the additional hydroxy metabolites GS-17791, GS-17792, and GS-17794, and concluded that high levels of polar and insoluble residues in mature tissues had not been adequately characterized, and additional data were required depicting the total terminal residue of radiolabeled atrazine in corn. Structures of atrazine and these chloro and hydroxy metabolites are indicated in Figure 1.

A subsequent review (DEB No. 5783, 5/3/90, M.S. Metzger) noted that all metabolites containing the intact triazine ring were now considered of toxicological concern, and data requirements should be revised such that all metabolites which contain the intact triazine ring are determined for all commodities for which atrazine is registered. The Agency subsequently issued a DCI, received by registrant in 10/90, which superceded the residue chemistry data requirements of all previous DCIs and any other agreements entered into with the Agency pertaining to such requirements. The DCI requirements included data depicting the total terminal residues of radiolabeled atrazine in corn, rye, sugarcane, sorghum, wheat, and pineapple. Registrant Ciba-Geigy's response to this DCI was reviewed and recommendations for conducting studies were provided (CBRS No. 9167, 1/22/92, M.S. Metzger).

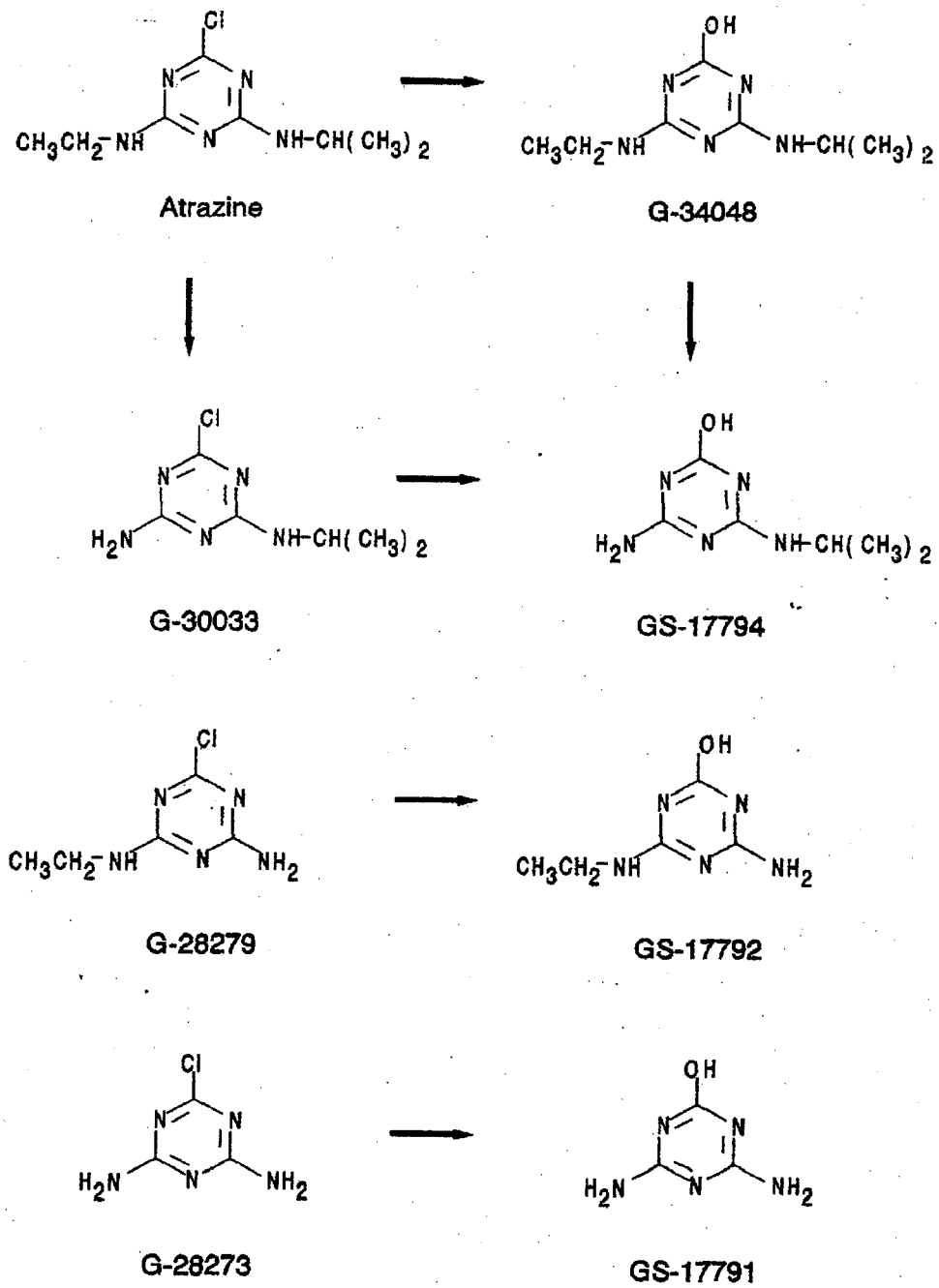


Figure 1. Atrazine, chloro (left), and hydroxy metabolites

The HED Metabolism Committee has addressed issues related to triazine chemicals (Memo, 8/7/92, M.S. Metzger). The Committee noted that in the absence of data on the toxicity of triazine metabolites, all metabolites containing a triazine ring with a substituent would be assumed toxicologically equivalent to the parent compound. Should data be reviewed and accepted which indicate that hydroxyatrazine is not carcinogenic, then the exposure assessment and tolerance expression for atrazine will include only parent and chloro metabolites. No analytical methods were available to determine total residues of metabolites containing triazine rings with substituents. Field studies using ^{14}C-atrazine would allow exposure assessment for total triazine ring residues as the total radioactive residue (TRR), because most of the radioactivity remains as triazine-ring containing metabolites; TRR will be assumed to represent all residues of concern. In addition to measuring TRR in the radiolabel field studies, the Registrant was also required to identify major components of the total radioactivity in crops. If possible, these data will be used to identify appropriate "marker" metabolites to use in developing analytical methods for enforcement purposes and for non-radiolabeled field residue data. The Metabolism Committee recommended that risk assessment should be conducted with the best data available for determining total residues of metabolites containing the triazine ring.

A hydroxyatrazine cancer study has been submitted, but review has not been completed. The previous conclusions of the Metabolism Committee therefore remain in effect at this time.

In response to these requirements, registrant Ciba-Geigy submitted the document:

^{14}C-Atrazine: Nature of the Residue in Sugarcane, Final Report Amendment No. 1, Hazelton Wisconsin, Inc., Project HWI 6117-181, October 18, 1993 (MRID 43016503).

NATURE OF THE RESIDUE, SUGAR

Field Procedures

Treatment and control plots of sandy loam soil, 4 x 20 ft, were prepared at Pan-Agricultural Labs, Madera, CA. Each plot was planted with one row of sugarcane (CP 721210 variety) on October 7, 1991. The treated plot was separated from the control plot by 370 ft, and was enclosed in a cyclone fence with a plastic barrier around it to control drift. The test substance was the 4L formulation, containing ^{14}C-atrazine, uniformly labeled in the aromatic ring at a specific activity of 14.5 μCi/mg and a radiochemical purity of 98%.

Sugarcane was treated with four applications of the test substance. The first application was pre-emergence broadcast spray, applied to the soil at 4 lb ai/A, on October 8, 1991, one day after planting. The second, third, and fourth applications were each at 2 lb ai/A. The second application was pre-emergence broadcast spray, applied to soil on November 15, 1991. The third application was post-emergence broadcast spray, applied May 2, 1992. The fourth treatment was post-emergence directed spray applied August 6, 1992, 137 days before final harvest on December 21, 1992. Control plots were treated in the same manner, but with unlabeled atrazine.

Control samples were always collected prior to treated samples, and were harvested and processed in the same fashion. Prior to the fourth application, one leaf from each of ten canes was collected from each plot. At final harvest, sugarcane was cut with a machete, leaves were stripped from canes, and canes divided into thirds. Samples collected in the field were placed in a cooler with ice, stored for up to four hours in the cooler, then transferred to a freezer where the temperature did not exceed -12°C. Samples were packed in dry ice and shipped to the performing laboratory, Hazelton Wisconsin, Madison, WI, or to Ciba-Geigy, Greensboro, NC for frozen storage. Upon arrival at Hazelton, samples were placed into frozen storage. Samples were stored at approximately -20°C when not being analyzed.

CBRS Comments, Sugar Field Procedures

In its response to the DCI of October 1990, Ciba-Geigy proposed to conduct a radiolabel field study with sugarcane treated at the maximum preemergence rate (CBRS 9167, 1/22/92, M.S. Metzger). The Second Round Review Residue Chemistry Chapter (10/18/88) noted that atrazine was registered for preemergence application (at planting or ratooning) to sugarcane at 2-4 lb ai/A, followed by an additional application at time of emergence and up to two interline postemergence applications as directed sprays prior to lay-by. A maximum of 10 lb ai/A may be applied to each sugarcane crop. A reasonable interval between lay-by and harvest would be 120-150 days, providing a built-in PHI.

For the field metabolism study, the total application was 10 lb ai/A, in two preemergence and two postemergence applications. The application one day after planting represented the maximum at planting rate, and the last application was near lay-by. The application pattern therefore represented the maximum rate per crop, the maximum rate at planting, the maximum number of post-emergence applications, and included an application during the last stage allowed by registered use. This combination of applications should result in extensive metabolism of the labeled chemical applied at planting, as well as the presence of early metabolites from the chemical applied near lay-by. The

8

combination of applications chosen was appropriate for a metabolism study.

Conclusion 1. Conclusions under this heading apply to the sugarcane field metabolism study:

Conclusion 1a: The application pattern represented the maximum rate per crop, the maximum rate at planting, the maximum number of postemergence applications, and included an application during the last stage allowed by registered use. The combination of applications chosen was appropriate for a metabolism study, and should produce a range of both early and later metabolites.

The previous review (CBRS 9167) noted that a sugarcane metabolism study had not been performed, and had the following comments:

A sufficient number of studies must be performed so that the likely variability in residues of total triazines in sugarcane commodities can be determined. Adequate geographical representation is necessary. We recommend that the registrant provide protocols prior to the initiation of these studies for CBRS review.

Conclusion 1b: The sugarcane field metabolism study was conducted at one site in CA. Geographic variability of residues is not known.

Laboratory Analysis

Total radioactive residues (TRR) of sugarcane samples were determined by combustion and liquid scintillation counting (LSC). Results are summarized in Table 1.

Table 1. TRR in sugarcane samples.

Sample	TRR, ppm
Pre-fourth application leaves	68.99
Final harvest leaves	24.22
Final harvest cane	2.09

The most extensive extraction protocol was Method I applied to sugarcane cane, shown in Figure 2. Plant samples were placed into methanol:water, 80:20, ground in a Tissumizer, and filtered. The methanol in the extract fractions was removed by rotary evaporation, and the extract was partitioned with equal volumes of chloroform. For sugarcane leaves, the extraction ended at this point.

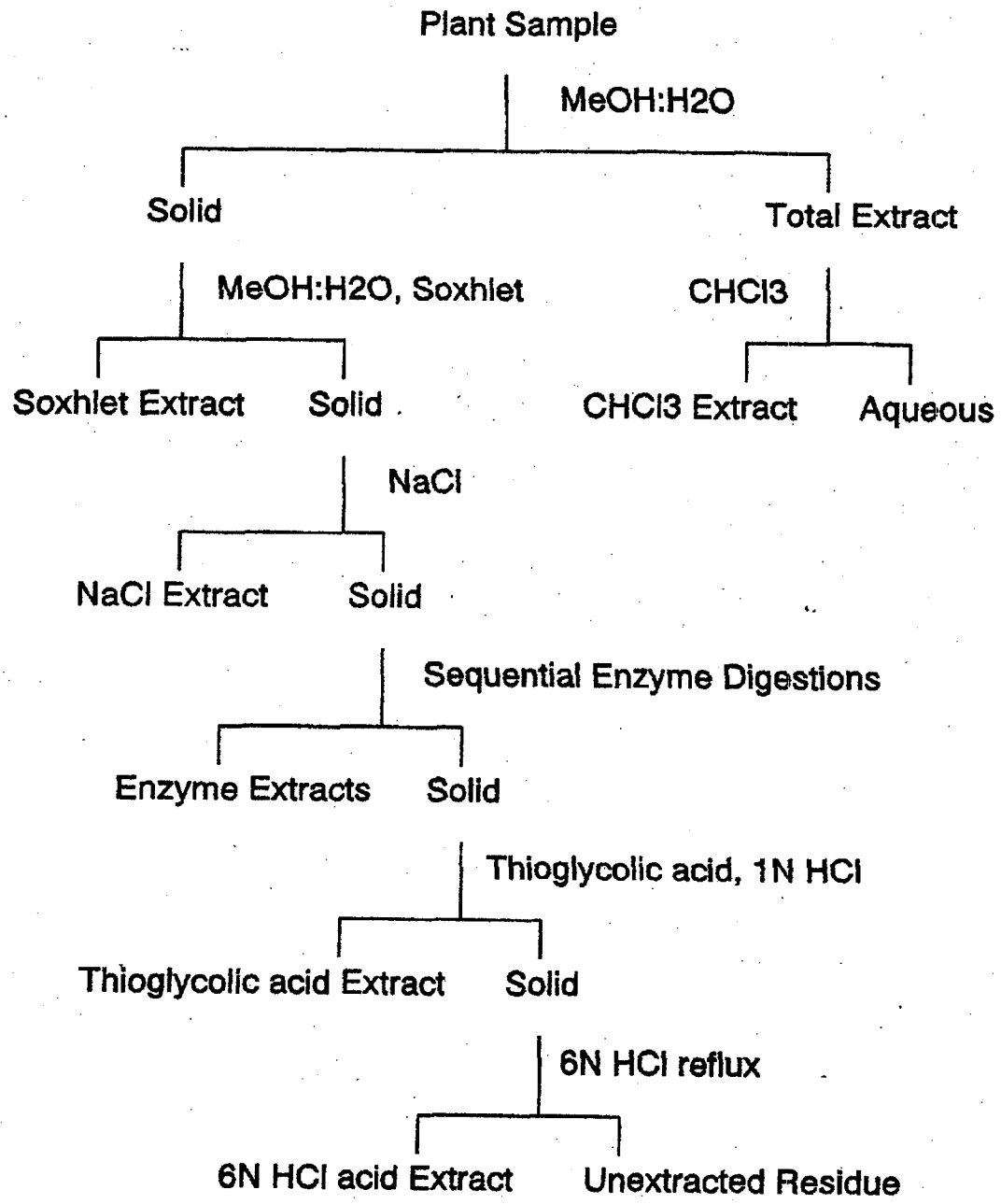


Figure 2. Extraction Method I for sugarcane cane.

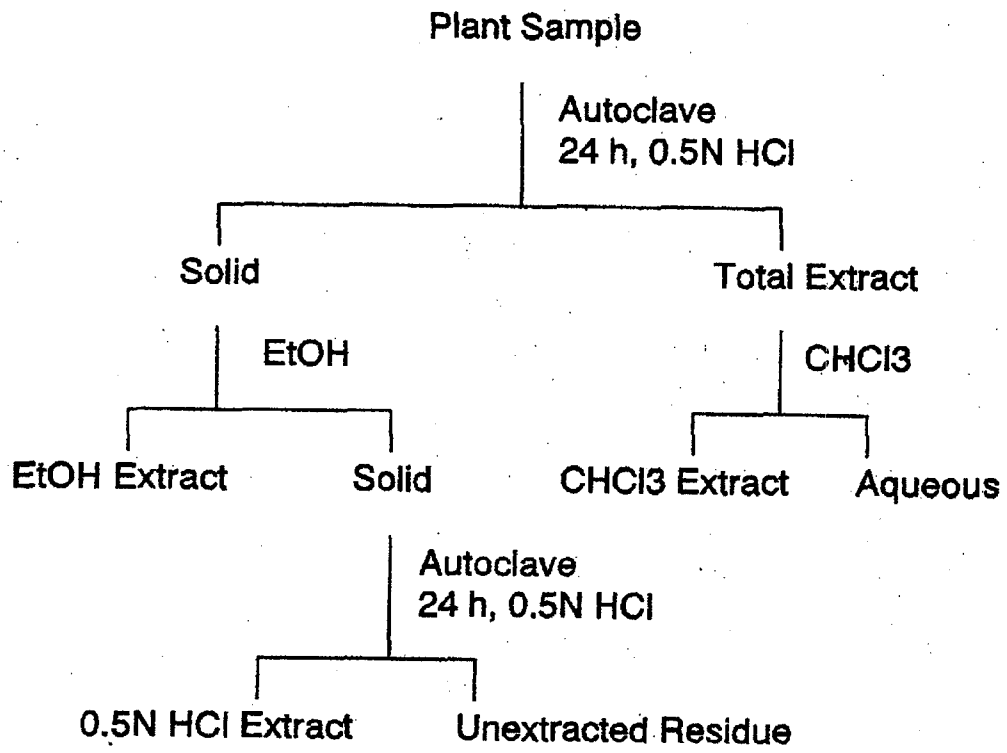


Figure 3. Extraction Method II for sugarcane.

For sugarcane cane, the solid after methanol:water extraction was placed in a Soxhlet apparatus, and soaked in water overnight. Methanol was added and residues were extracted by Soxhlet for 4 h. The Soxhlet extract was concentrated by rotary evaporation and redissolved in water. After Soxhlet extraction, the remaining nonextracted residue was refluxed in 1% sodium chloride solution for 4 h, cooled, and filtered. The filtrate was concentrated and redissolved in water.

The nonextracted residue after sodium chloride extraction was resuspended in buffer and treated successively by β-glucosidase, α-amylase, protease, and cellulase. As will be seen below, treatment by all enzymes combined released only a small portion of the TRR. The nonextracted residue following enzyme treatments was added to 20 ml of 1N HCl with 4 g thioglycolic acid, refluxed for 6 h, cooled, and filtered. The filter cake was washed with 95% ethanol, and the thioglycolic acid and ethanol extracts were concentrated separately. The nonextracted residue following thioglycolic acid treatment was added to 6N HCl and refluxed for 4 h, cooled, and filtered. The remaining unextracted residue was analyzed by combustion and liquid scintillation counting.

Final harvest leaves and cane were also extracted by Method II, shown in Figure 3. Plant samples were incubated in 0.5N HCl in an autoclave for approximately 24 h, filtered, and rinsed with water. The extract was neutralized with ammonium hydroxide and partitioned three times with equal volumes of chloroform. The chloroform layers were combined and partitioned once with an equal volume of water. The water layer was combined with the aqueous fractions.

Under Method II, a subsample of the initial residue was extracted with 95% ethanol and shaken for about 2 h. The mixture was filtered, rinsed with 95% ethanol, and the filtrates were combined. The remaining unextracted residue was incubated in 0.5N HCl for about 24 h in the autoclave. The mixture was filtered, rinsed with water, and the filtrates were combined. The remaining unextracted residue was analyzed by combustion and liquid scintillation counting. For both Methods I and II, radioactivity in aqueous and organic fractions was determined by liquid scintillation counting. The distributions of TRR with Methods I and II are summarized in Tables 2 and 3:

Table 2. Extraction of TRR from sugarcane samples by Method I.

Fraction	% TRR (ppm) for [100% TRR]:		
	Pre-fourth application leaves [69.0 ppm]	Final harvest leaves [24.2 ppm]	Final harvest cane [2.09 ppm]
Chloroform	3.9 (2.66)	3.1 (0.75)	3.3 (0.07)
Aqueous	59.2 (40.8)	47.9 (11.6)	56.9 (1.19)
Soxhlet			5.0 (0.10)
NaCl			10.6 (0.22)
Combined Enzyme			1.8 (0.04)
Thioglycolic acid			11.3 (0.24)
Ethanol wash			1.0 (0.02)
6N HCl			0.7 (0.02)
Unextracted	34.0 (23.5)	39.5 (9.57)	1.0 (0.02)

Table notes: Method I is shown in Figure 2. Soxhlet and subsequent treatments were performed only on cane.

Table 3. Extraction of TRR from sugarcane samples by Method II.

Fraction	% TRR (ppm) for [100% TRR]:	
	Final harvest leaves [24.2 ppm]	Final harvest cane [2.09 ppm]
Chloroform	0.5 (0.12)	1.0 (0.02)
Aqueous	76.8 (18.6)	78.0 (1.63)
Ethanol	5.7 (1.38)	3.0 (0.06)
0.5N HCl	1.5 (0.36)	2.3 (0.05)
Unextracted	9.5 (2.30)	10.7 (0.22)

Table notes: Method II is shown in Figure 3.

Residue Identification

Fractions extracted by Method I were further analyzed to identify residues. Organic and aqueous fractions were analyzed by

2-dimensional thin layer chromatography (2-D TLC) using silica gel plates. The solvent in the first dimension was chloroform:methanol:formic acid:water (75:20:4:2), and the solvent in the second dimension was butanol:acetic acid:water (80:10:10). One-dimensional TLC was also used to purify radioactive residue fractions and to verify the results of other analyses.

Organic (chloroform) fractions from extraction Method I were analyzed by 2-D TLC and by reverse phase HPLC using a YMC-Pack ODS column, eluted with gradients in 1% acetic acid in water and 1% acetic acid in acetonitrile (HPLC Method I). Peaks from TLC and HPLC were assigned based on similar mobilities with standards. Residues identified in the organic fractions were parent atrazine, the chloro metabolites G-28279 and G-30033, and GS-12517 and CGA-101248 (see Figure 4 for structures). Assignment of known residues was consistent between TLC and HPLC, and unidentified peaks each represented less than 1.0% TRR.

Aqueous fractions were analyzed by Aminex A-4 cation exchange chromatography, and by HPLC, using HPLC Method I or using a Whatman Partisil PAC column in series with a Phenomenex SCX column, eluted with gradients in acetonitrile:0.1 M ammonium formate (94:6) and acetonitrile:0.1 M ammonium formate:methanol (70:20:10) (HPLC Method VI). Peaks were assigned based on similar mobilities with standards. Residues identified were the four hydroxy compounds (see right side of Figure 4), G-28273, GS-12517, CGA-101428, and unknowns designated Aminex Peaks 4 and 7. The identity of the known compounds was confirmed by comparing mobilities of the Aminex peaks with those of standards during HPLC.

Peaks assigned to G-28273, GS-12517, and CGA-101428 were purified by HPLC and identities were confirmed by gas chromatography-mass spectrometry and comparison with standards. Aminex Peak 4 was purified by HPLC, and mass spectrometry indicated that it was a glucose-thiolactic acid conjugate of atrazine. Hydrolysis of Peak 4 with 0.5N HCl in an autoclave for 24 h produced G-34048, hydroxyatrazine, further indicating that the unknown was an atrazine conjugate. The presence of glucose was supported by a change in the chromatographic profile following treatment with ß-glucosidase, and by NMR data. Based on metabolism in sorghum, an atrazine lanthionine conjugate was synthesized; Aminex Peak 7 was assigned as this conjugate based on similar chromatographic behavior. Figure 5 shows the full structures of these conjugates; Figure 4 shows them in a proposed metabolism scheme with abbreviated structures. Registrant proposed that the structures on the left side of Figure 4 were produced by conjugation of glutathione with atrazine, and further metabolism or rearrangement of the glutathione moiety through intermediates that were not isolated.

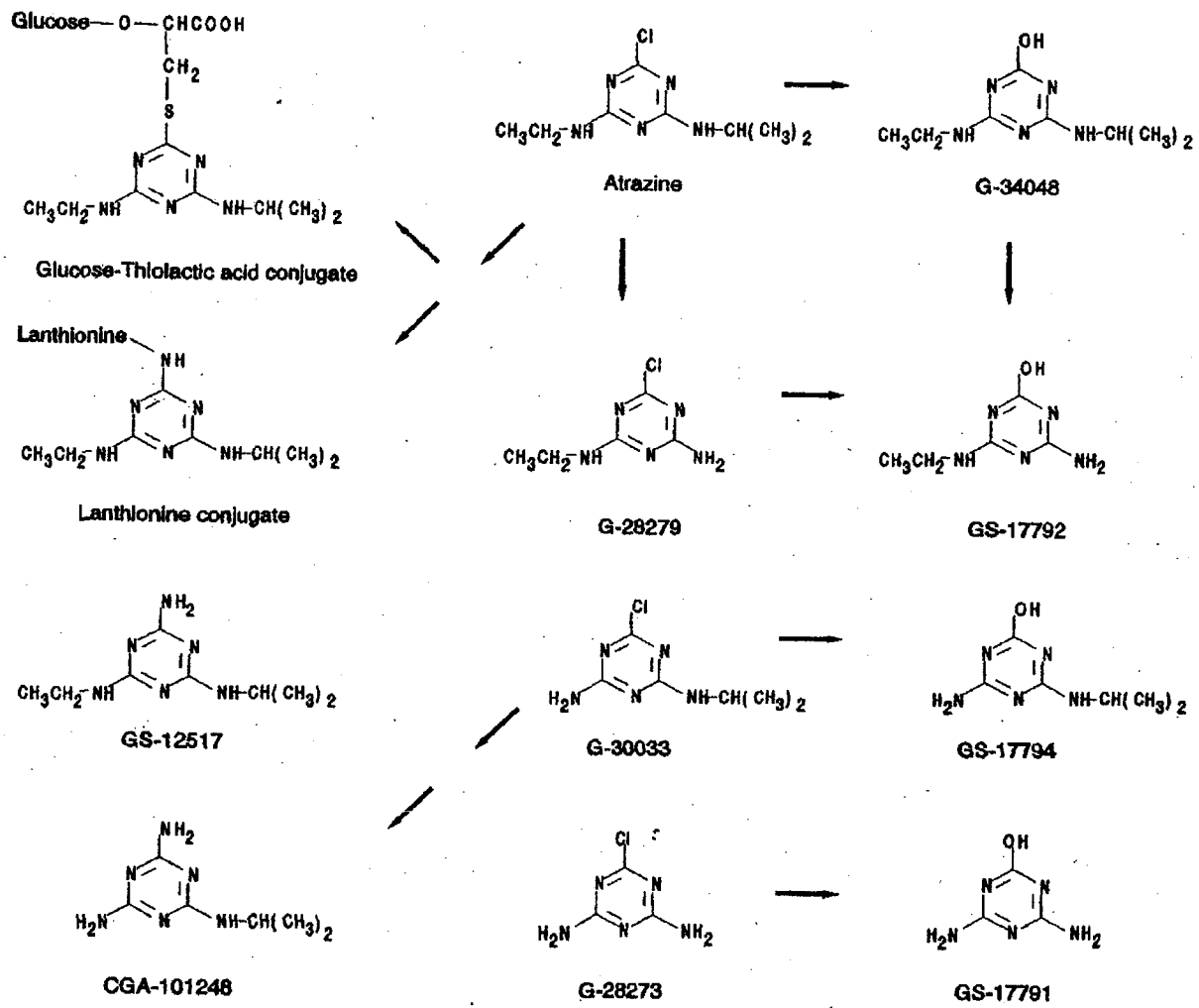


Figure 4. Identified atrazine metabolites in sugarcane.

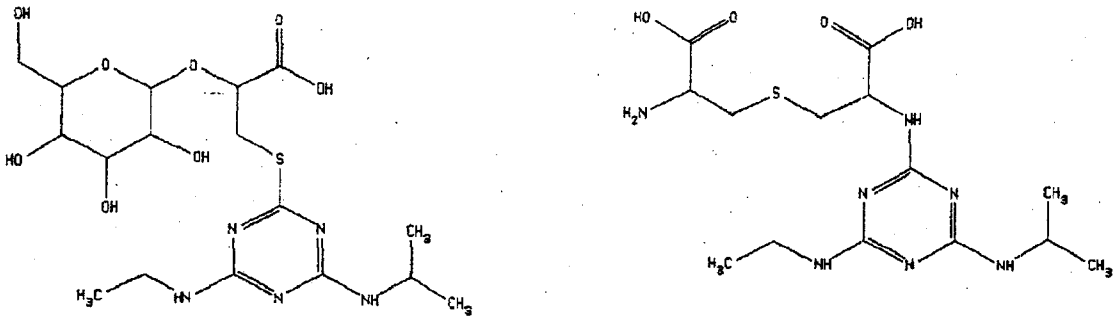


Figure 5. Full structures for atrazine conjugates with glucose-thiolactic acid (left) and lanthionine (right)

The aqueous fractions produced by Extraction Method II, acid autoclave, were also analyzed; other fractions each represented less than 6% TRR and were not further evaluated. The major residues identified by Aminex cation exchange chromatography were three hydroxy compounds and cyanuric acid (see Figure 6 for structures). A small amount of CGA-101248 (0.3% TRR) was observed in the cane sample only, after extraction by Method II. Identifications of cyanuric acid, G-34048, GS-17794, and GS-17792 were confirmed by chromatography of each assigned Aminex peak using HPLC Methods I or VI, and comparison with standards.

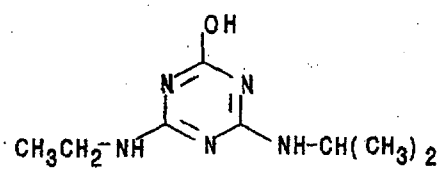
Standards or residues isolated by Extraction Method I were treated in 0.5N HCl for 24 h in an autoclave, and the products were determined. Results are summarized in Table 4. Registrant concluded that Method II should convert atrazine or any of its metabolites to one or more of the residues in Figure 6:

Table 4. Conversion of individual residues after acid autoclave treatment.

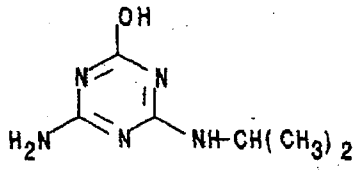
Initial residue	Product
G-28273	Cyanuric acid
G-34048	G-34048
GS-17794	Cyanuric acid and GS-17794, each ≈ 40% TRR
GS-17791	Cyanuric acid
GS-12517	G-34048
Atrazine Glucose-thiolactic acid conjugate	G-34048

Table note: See Figures 4 through 6 for structures.

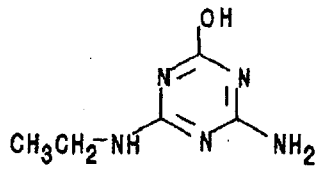
16



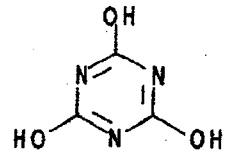
G-34048



GS-17794



GS-17792



Cyanuric Acid

Figure 6. Residues detected after Extraction Method II.

Table 5 provides a comparison of residues assigned under Extraction Methods I and II. Residues were assigned to leaf samples extracted by Method I based on analysis of the chloroform and methanol:water fractions. With cane, the extended Method I treatment (see Figure 2) released additional residues in aqueous fractions, and a greater part of the TRR was identified as known compounds.

The Registrant provided data on cane samples extracted by Method I in January 1993, a month after harvest, and May 1993, within three months of the termination of laboratory work. The aqueous fraction from each sample was analyzed by Aminex A-4 cation exchange chromatography. The chromatographic profiles and quantity of radioactive residues were comparable for the two samples, indicating acceptable storage stability of residues during the course of laboratory analysis.

CBRS Comments, Residue Identification in Sugarcane

Conclusion 1c: Atrazine undergoes extensive metabolism in sugarcane. The Registrant has identified chloro, hydroxy, amino, and conjugated metabolites, each containing an intact triazine ring (for details, see Table 5 and Figures 4 through 6 of this review). Using Extraction Method I, residues identified represented 26.9% TRR in final harvest leaves, and 52.5% TRR in final harvest cane. Atrazine and other chloro compounds combined represented 5% TRR in final harvest cane, and less than 2% TRR in final harvest leaves.

Conclusion 1d: With Extraction Method II, an acid autoclave procedure, approximately two-thirds of the TRR in final harvest leaves and cane was converted to cyanuric acid and the atrazine hydroxy metabolites G-34048, GS-17794, and GS-17792. This observation indicates that the position of the HED Metabolism Committee, that TRR should represent total residues containing the triazine ring, is a reasonable assumption.

Table 5. Assignment of Residues by Extraction Method I or II.

Residue	Pre-fourth application leaves:	Final harvest leaves		Final harvest cane	
	Method I, % TRR (ppm)	Method I, % TRR (ppm)	Method II, % TRR	Method I, % TRR (ppm)	Method II, % TRR
Atrazine	0.2 (0.162)	<0.1 (0.017)		0.3 (0.007)	
G-30033	0.5 (0.356)	0.1 (0.027)		1.4 (0.029)	
G-28279	0.2 (0.145)	<0.1 (0.015)		0.4 (0.009)	
G-28273	8.2 (5.694)	1.7 (0.426)		3.0 (0.063)	
[Total chloros]	[9.1]	[1.8]		[5.1 (0.108)]	
GS-12517	1.0 (0.687)	1.5 (0.357)		6.5 (0.136)	
CGA-101248	2.2 (1.508)	2.4 (0.593)		2.0 (0.042)	0.3
G-34048	0.7 (0.484)	0.3 (0.083)	26.1	2.4 (0.050)	31.7
GS-17794	8.5 (5.860)	7.1 (1.727)	14.7	10.1 (0.211)	12.3
GS-17792	0.9 (0.604)	0.6 (0.141)	1.1	0.8 (0.017)	1.7
GS-17791	1.8 (1.216)	1.0 (0.251)		0.9 (0.019)	
Cyanuric acid			24.9		20.5
Glucose-thiolactic acid conjugate	11.1 (7.678)	11.8 (2.852)		6.6 (0.138)	
Lanthionine conjugate	1.4 (0.985)	0.4 (0.103)		18.1 (0.379)	
Total Assigned	36.7 (25.379)	26.9 (6.592)	66.8	52.5 (1.099)	66.5

Table notes: See Figures 4 through 6 for structures. Pre-fourth application leaves were extracted by Method I only. Values for ppm are shown for Method I only. Blank spaces indicate residues not detected.

CBRS has previously determined anticipated residues for atrazine, for combined residues of parent and chloro metabolites; anticipated residues for sugarcane cane were 0.13 ppm (DEB 3688-3703, 9/14/88, M.S. Metzger). In the present submission, parent and chloro metabolites represented 0.108 ppm in cane; TRR in cane was 2.09 ppm (Tables 5 and 1, respectively). We have previously reported these values to SRRD, noting at that time that review of the metabolism study was not completed (Memo, 1/26/94, J. Abbotts). The present submission was a metabolism study in a single location, while the anticipated residues were determined from several field trials. Because there is little difference between the anticipated residue value and the combined residues of parent and chloro residues in the present submission, there is no need to revise anticipated residues previously determined. However, the present submission provides a ratio for TRR:parent and chloro residues (2.09:0.108). Applying this ratio to anticipated residues for parent and chloro metabolites (0.13 ppm) gives an anticipated TRR of 2.52 ppm. In accordance with the present position of the HED Metabolism Committee that exposure assessment should be conducted on the basis of TRR, 2.52 ppm should be used for total residues containing the triazine ring.

Conclusion 1e: In the present submission, combined residues of parent and chloro metabolites in cane were 0.108 ppm. CBRS previously determined combined anticipated residues of parent and chloro metabolites of 0.13 ppm based on field trial data (DEB 3688-3703, 9/14/88, M.S. Metzger). This value for anticipated residues should still be considered valid, because the present submission was a metabolism study in a single location. However, in accordance with the present position of the HED Metabolism Committee that exposure assessment should be conducted on the basis of TRR, 2.52 ppm should be used for sugarcane cane for total residues containing the triazine ring, based on data in the present submission. SRRD has previously been advised of the residue data for sugarcane reported in the present submission (Memo, 1/26/94, J. Abbotts).

Conclusion 1f: Further data on field metabolism in sugarcane are not required.

NATURE OF THE RESIDUE, FIELD ROTATIONAL CROPS

Field Procedures

To support reregistration of atrazine on rotational crops, registrant also submitted the following document:

^{14}C-Atrazine: Uptake and Metabolism of Atrazine in Field Rotational Crops Following Corn and Sorghum Treated at a Rate of 3.0 lb ai/A, Hazelton Wisconsin, Inc., Project HWI 6117-183, November 12, 1993 (MRID 43016505).

The Registrant has previously submitted data on field metabolism studies in corn and sorghum in IL, MS, and NY; these data are considered acceptable (CBRS 10980, 6/6/93, J. Abbotts; CBRS 13059, 5/22/95, J. Abbotts). The same fields were planted in rotational crops wheat, spinach, and garden beets. The present submission thus represents the results of field rotational crop metabolism studies. The registrant noted that data on wheat were relevant to registered use for wheat as a target crop after chemical treatment of fallow land.

Plots 6 x 14 ft were prepared at Ciba-Geigy research stations in MS (silty loam soil), IL (clay loam), and NY (loam, 12 x 14 ft). One half of each plot was planted with corn, and the other half with sorghum. At each location, control plots were established at a distance from the radioactive plot to prevent contamination.

The test substance was the 4L formulation, containing ^{14}C-atrazine, uniformly labeled in the aromatic ring at a specific activity of 20.9 µCi/mg and a radiochemical purity of 98%. The 4L formulation was applied post-emergence by foliar spray using ground equipment at the maximum label rate of 3 lb ai/A. Control plots at each location were treated in the same manner, but with unlabeled atrazine. In its Overview document on corn and sorghum (MRID 42547115), the Registrant noted that the maximum use rate on corn and sorghum has been reduced to 2.5 lb ai/A (CBRS 10980, 6/6/93, J. Abbotts).

Following harvest of corn and sorghum, or shortly before harvest (NY wheat), soil at each plot was prepared for planting by hand implements. The MS plot was planted with winter wheat, which failed to germinate. The plot was subsequently planted with spring wheat, garden beets, and spinach 9 mo after treatment. The IL plot was planted with winter wheat 3 mo after treatment, and beets and spinach 10 mo after treatment. The NY plot was planted with winter wheat 3 mo after treatment, and beets and spinach 10 mo after treatment.

Control samples were always collected prior to treated samples, and were harvested and processed in the same fashion. Samples were collected by hand and stored in a freezer at each site, where the storage temperature did not exceed -5°C. Samples were packed in dry ice and shipped to the performing laboratory, Hazelton Wisconsin, Madison, WI. Upon arrival at Hazelton, samples were placed into frozen storage. Samples were stored at approximately -20°C when not being analyzed. Crops were planted and samples collected according to the schedule in Table 6:

Table 6. Schedule for treatment and rotational crops.

Event/Sample	IL Date	MS Date	NY Date
Application to corn and sorghum	06/10/91	06/13/91	06/12/91
Planting, winter wheat	09/09/91		09/11/91
Planting, spring wheat		03/28/92	
Planting, spinach	04/08/92	03/28/92	04/10/92
Planting, beets	04/08/92	03/28/92	04/10/92
60 DAP wheat forage	11/08/91	05/27/92	11/11/91
210/270 DAP wheat forage	04/07/92		06/10/92
Mature spinach	06/03/92	05/27/92	06/10/92
Mature beets and leaves	07/14/92	06/16/92	09/10/92
Mature wheat grain and straw	07/10/92	07/19/92	07/13/92

Table note: DAP = days after planting.

Laboratory Analysis

Total radioactive residues (TRR) of plant samples were determined by combustion and liquid scintillation counting (LSC). Results are summarized in Table 7:

Table 7. TRR in rotational crop samples.

Sample	TRR, ppm, at:		
	IL	MS	NY
60 DAP wheat forage	0.359	0.087	0.313
210 DAP wheat forage	0.227		
270 DAP wheat forage			0.164
Mature wheat straw	0.335	0.270	0.329
Mature wheat grain	0.044	0.048	0.071
Mature spinach leaves	0.217	0.070	0.112
Mature beet tops	0.081	0.061	0.071
Mature beet roots	0.019	0.030	0.042

Table notes: DAP = days after planting.
Spring wheat was grown in MS and matured in 113 days.

Plant samples were extracted by Method I, similar to Method I for sugarcane samples (see Figure 2 here). Plant samples were first extracted into methanol:water. Methanol was removed by rotary evaporation, and the extract was partitioned with equal volumes of chloroform. For most rotational crop samples, the extraction ended at this point.

Winter wheat grain from NY and beet roots from IL were extracted more extensively, similar to the full extraction Method I for sugarcane (Figure 2). Variations from the procedure for sugarcane were that sequential enzyme treatment was limited to α-amylase and protease, and the penultimate treatment was reflux in 1N HCl, without thioglycolic acid.

Mature samples of wheat, spinach, and beets from IL were also extracted by Method II, the acid autoclave extraction used with sugarcane (Figure 3). For both Methods I and II, radioactivity in aqueous and organic fractions was determined by liquid scintillation counting. The distributions of TRR with Method I are summarized in Tables 8 and 9, and distributions with Method II are summarized in Table 10:

Table 8. Extraction of TRR from wheat samples by Method I.

Fraction	% TRR (ppm) for [100% TRR in ppm]:										
	IL				MS (spring wheat)			NY			
	60 DAP Forage [0.359]	210 DAP Forage [0.227]	Mature Straw [0.335]	Mature Grain [0.044]	60 DAP Forage [0.087]	Mature Straw [0.270]	Mature Grain [0.048]	60 DAP Forage [0.313]	270 DAP Forage [0.164]	Mature Straw [0.329]	Mature Grain [0.071]
Chloroform	64.2	55.8	11.1	1.4	30.4	4.9	1.1	57.1	42.1	11.4	0.4 (<0.001)
Aqueous	19.4	27.4	30.7	17.9	42.5	25.3	10.1	20.1	35.5	62.3	18.6 (0.013)
Soxhlet											6.8 (0.005)
NaCl											38.0 (0.027)
Amylase											8.6 (0.006)
Protease											9.3 (0.007)
1N HCl											3.5 (0.002)
6N HCl											1.2 (<0.001)
Unextracted	17.1	14.8	56.7	88.0	26.5	61.1	82.0	16.0	18.5	32.3	2.8 (0.002)

Table notes: DAP = days after planting. Method I is outlined in Figure 2.
Soxhlet and subsequent treatments were performed only on NY mature wheat; ppm values are provided for this sample.

24

Table 9. Extraction of TRR from spinach and beet samples by Method I.

Fraction	% TRR (ppm) for [100% TRR in ppm]:								
	IL			MS			NY		
	Spinach Leaves [0.217]	Beet Tops [0.081]	Beet Roots [0.019]	Spinach Leaves [0.070]	Beet Tops [0.061]	Beet Roots [0.030]	Spinach Leaves [0.112]	Beet Tops [0.071]	Beet Roots [0.042]
Chloroform	70.9	39.1	2.7 (<0.001)	18.4	8.5	1.4	40.6	23.3	3.4
Aqueous	25.1	37.9	58.1 (0.011)	46.9	27.6	45.1	35.6	34.8	48.5
Soxhlet			5.0 (<0.001)						
NaCl			2.7 (<0.001)						
Amylase			13.5 (0.003)						
Protease			1.6 (<0.001)						
1N HCl			2.0 (<0.001)						
6N HCl			7.2 (0.001)						
Unextracted	9.0	27.2	6.5 (0.001)	34.2	69.4	61.9	31.0	45.5	43.0

Table notes: DAP = days after planting. Method I is outlined in Figure 2.
Soxhlet and subsequent treatments were performed only on IL beets; ppm values are provided for this sample.

Table 10. Extraction of TRR from IL samples by Method II.

Fraction	% TRR for [100% TRR in ppm]:				
	Mature Wheat Straw [0.335]	Mature Wheat Grain [0.044]	Mature Spinach Leaves [0.217]	Mature Beet Tops [0.081]	Mature Beet Roots [0.019]
Aqueous	72.2	78.0	98.2	85.3	82.7
Chloroform	0.3	0.8	0.4	0.1	0.7
Ethanol	4.1	3.5			2.8
0.5N HCl	2.3	2.0			1.9
Unextracted	15.1	11.5	2.4	7.3	10.5

Table notes: Method II is outlined in Figure 3. This treatment was performed only on IL mature samples; the second acid autoclave treatment was not applied to spinach leaves and beet tops.

Residue Identification

Extracted fractions were further analyzed to identify residues, using TLC, HPLC, and Aminex column chromatography methods similar to those applied for identification of residues in sugarcane. Residues identified in rotational crops were the same as those identified in sugarcane using extraction Method I (see Figure 4) or Method II (see Figure 6), respectively. Tables 11 and 12 summarize the residues assigned using extraction Method I, and Table 13 provides a comparison of residues assigned to the IL samples with Methods I and II.

To examine storage stability, IL samples were extracted by Method I near the beginning (11/92-2/93) and end (9/93-11/93) of the laboratory analysis period. Organic fractions from 60 DAP wheat forage, spinach leaves, and beet tops were analyzed by 2-D TLC. Aqueous fractions of mature wheat straw, wheat grain, spinach leaves, beet tops, and beet roots were analyzed by Aminex ion exchange chromatography. The chromatographic profiles and quantity of radioactive residues were comparable for the early and later extractions for all samples, indicating acceptable storage stability of residues during the course of laboratory analysis.

Table 11. Assignment of Residues to wheat samples extracted by Method I.

Residue	% TRR for [100% TRR in ppm]:										
	IL				MS (spring wheat)			NY			
	60 DAP Forage [0.359]	210 DAP Forage [0.227]	Mature Straw [0.335]	Mature Grain [0.044]	60 DAP Forage [0.087]	Mature Straw [0.270]	Mature Grain [0.048]	60 DAP Forage [0.313]	270 DAP Forage [0.164]	Mature Straw [0.329]	Mature Grain [0.071]
Atrazine	39.5	24.6	1.5		3.2	0.1		30.9	9.1	1.6	
G-30033	20.0	17.6	4.0		7.6	0.1		21.5	26.4	6.0	
G-28279	2.0	8.8	1.3		0.1	0.2		1.6	1.9	0.7	
G-28273	3.3	9.7	7.4	2.6	14.2	3.1	1.7	3.3	14.0	22.0	7.8
[Total chloros (ppm)]	[64.8 (0.233)]	[60.7 (0.138)]	[14.2 (0.046)]	[2.6 (0.001)]	[25.1 (0.022)]	[3.5 (0.009)]	[1.7 (0.001)]	[57.3 (0.179)]	[51.4 (0.083)]	[30.3 (0.099)]	[7.8 (0.006)]
G-34048	0.5	0.6	0.5			0.4			0.2	0.4	0.1
GS-17794	3.7	2.3	2.8	0.8	4.8	2.6	0.5	2.3	1.5	3.5	2.5
GS-17792		0.4	0.7	0.5	1.5	3.8			<0.1	1.5	0.5
GS-17791	1.4	1.2	0.9	2.5	2.1		0.8	0.5	1.4	2.2	8.3
GS-12517						0.1					
CGA-101248			0.6	0.4	3.4	3.5	0.4			1.0	0.2
Glucose-thiolactic acid conjugate	0.2	0.5	1.4	0.3	0.7	0.3	1.0	0.2	0.5	1.1	0.5
Lanthionine conjugate	0.6	0.3	0.6	0.2	0.4	1.2	0.3	0.2	0.4	0.8	0.4
Total Assigned	71.2	66.0	21.7	7.3	38.0	15.4	4.7	60.5	55.4	40.8	20.3

Table notes: See Figures 4 and 5 for structures. Blank spaces indicate residues not detected. Extended extraction protocol used on NY grain.

27

Table 12. Assignment of Residues to spinach and beet samples extracted by Method I.

Residue	% TRR for [100% TRR in ppm]:								
	IL			MS			NY		
	Mature Spinach Leaves [0.217]	Mature Beet Tops [0.081]	Mature Beet Roots [0.019]	Mature Spinach Leaves [0.070]	Mature Beet Tops [0.061]	Mature Beet Roots [0.030]	Mature Spinach Leaves [0.112]	Mature Beet Tops [0.071]	Mature Beet Roots [0.042]
Atrazine	17.8	22.6	1.7	0.3		0.1	4.8	7.3	0.5
G-30033	48.7	10.9	2.1	13.5		0.4	31.1	14.1	2.4
G-28279	0.3	0.5	0.5	0.1		0.1	1.1	0.5	0.2
G-28273	6.8	12.2	4.4	20.1	1.9	1.5	10.0	7.7	3.6
[Total chloros (ppm)]	[73.6 (0.160)]	[46.2 (0.037)]	[8.7 (0.002)]	[34.0 (0.024)]	[1.9 (0.001)]	[2.1 (0.001)]	[47.0 (0.053)]	[29.6 (0.021)]	[6.7 (0.003)]
G-34048	0.9	1.0	8.7	0.1	0.5	3.6	0.2	0.1	
GS-17794	1.1	3.5	4.1	2.0	2.9	4.6	1.8	3.9	3.0
GS-17792	0.2	0.4	0.7	1.5	1.0	1.0	0.1	0.6	
GS-17791	0.3	1.3	0.9	0.7	0.7	1.4	2.3		0.8
GS-12517			0.1						
CGA-101248		0.5	1.8	5.5	4.0	2.4		1.4	
Glucose-thiolactic acid conjugate	0.3	0.3	0.2	0.5	0.2	0.1	0.4	0.2	<0.1
Lanthionine conjugate	0.4	0.4	0.8	0.8	0.3	0.9	0.4	0.8	0.2
Total Assigned	76.8	53.6	26.0	45.1	11.5	16.1	52.2	36.6	10.7

Table notes: See Figures 4 and 5 for structures. Blank spaces indicate residues not detected. Extended extraction protocol used on IL beet roots.

Table 13. Assignment of Residues to IL samples by Method I or II.

Residue	% TRR assigned by Method for:									
	Mature Wheat Straw		Mature Wheat Grain		Mature Spinach Leaves		Mature Beet Tops		Mature Beet Roots	
	I	II	I	II	I	II	I	II	I	II
Atrazine	1.5				17.8		22.6		1.7	
G-30033	4.0				48.7		10.9		2.1	
G-28279	1.3				0.3		0.5		0.5	
G-28273	7.4		2.6		6.8		12.2		4.4	
G-34048	0.5	5.5		1.1	0.9	17.3	1.0	19.1	8.7	16.0
GS-17794	2.8	7.0	0.8	0.6	1.1	24.4	3.5	10.4	4.1	8.5
GS-17792	0.7	2.3	0.5	1.2	0.2	1.9	0.4	0.5	0.7	3.1
GS-17791	0.9		2.5		0.3		1.3		0.9	
GS-12517									0.1	
CGA-101248	0.6		0.4				0.5		1.8	
Cyanuric acid		41.5		49.4		42.6		34.9		37.9
Glucose-thiolactic acid conjugate	1.4		0.3		0.3		0.3		0.2	
Lanthionine conjugate	0.6		0.2		0.4		0.4		0.8	
Total Assigned	21.7	56.3	7.3	52.3	76.8	86.2	53.6	64.9	26.8	65.5

Table notes: See Figures 4 through 6 for structures. Blank spaces indicate residues not detected.

CBRS Comments, Residue Identification in Rotational Crops

Conclusion 2. The conclusions under this heading apply to the rotational crop field metabolism studies:

Conclusion 2a: Atrazine undergoes extensive metabolism in the rotational crops wheat, spinach, and garden beets. The Registrant has identified chloro, hydroxy, amino, and conjugated metabolites, each containing an intact triazine ring (for details, see Tables 11 and 12 and Figures 4 through 6 of this review). Using Extraction Method I, residues identified were as low as 4.7% TRR in a mature grain sample, and as high as 76.8% TRR in a mature spinach sample. The distribution of residues varied with crop, with commodity of the same crop, and with geographical location. Atrazine and other chloro compounds combined represented less than 2% TRR in samples of mature grain and mature beet tops, and up to 74% TRR in a sample of mature spinach leaves.

Conclusion 2b: When Extraction Method II, an acid autoclave procedure, was applied to samples from IL, from 52% TRR to 86% TRR in mature rotational crops was converted to cyanuric acid and the atrazine hydroxy metabolites G-34048, GS-17794, and GS-17792. These observations indicate that the position of the HED Metabolism Committee, that TRR should represent total residues containing the triazine ring, is a reasonable assumption for rotational crops.

Under the present position of the HED Metabolism Committee, TRR represents the residues of concern. If data are reviewed and accepted which indicate that hydroxyatrazine is not carcinogenic, then the tolerance expression for atrazine will include only parent and chloro metabolites (see Reregistration Requirements section here). However, Toxicology Branch has indicated that in the absence of data to the contrary, hydroxyatrazine and other metabolites will be considered toxicologically equivalent to parent for non-cancer chronic endpoints (M. Beringer, personal communication). Data on TRR therefore will still be necessary for exposure assessment.

In addition to the present submission, the Registrant has previously submitted field metabolism data on corn and sorghum in IL, MS, and NY, and these studies have been accepted (CBRS 10980, 6/6/93, J. Abbotts; CBRS 13059, 5/22/95, J. Abbotts). The Registrant has identified 12 specific metabolites in sugarcane (see Figure 4 here), and the same metabolites have collectively been identified in rotational crops (Tables 11 and 12). The study in corn identified parent, chloro, and hydroxy metabolites shown in Figure 4, and the study in sorghum identified these residues and the lanthionine conjugate in addition. All studies also provided data on TRR.

The work to date indicates that atrazine metabolism in plants is extensive, individual metabolites generally represent small portions of TRR in mature crop commodities, and TRR is a reasonable representation of total triazine ring residues. Further work to identify additional metabolites in rotational crops would not be expected to alter these overall conclusions, nor to result in simpler tolerance expressions. The data on rotational crops provided are sufficient to reach the following conclusion:

Conclusion 2c: Metabolic pathways are similar for rotational crops and primary crops, at least for early metabolites. No further data are required for field metabolism studies of rotational crops.

Under Chemistry Branch Guidance on How to Conduct Studies on Rotational Crops (2/24/93, E. Zager and D. Edwards), if levels of residues to be regulated are ≥0.01 ppm in the rotational crop for the maximum plantback interval, then limited field trials are required on representative crops at two sites per crop for each of the crop groups root and tuber vegetables, leafy vegetables, and cereal grains, for a total of six trials. The field metabolism studies in the present submission provide metabolism data from three sites per crop for each of the three required crop groups. Maximum plantback intervals were 9 months for wheat, and 10 months for each of spinach and beets (see Table 6). Data were provided on 12 individual metabolites, as well as TRR. These data are sufficient to conclude that:

Conclusion 2d: The field metabolism studies are sufficient to meet the requirements for limited field rotational crop studies.

Under guidance for rotational crops (Ibid.), if detectable residues are observed in the limited field trials, then rotational crop tolerances are required on all crop groups which registrant intends to support. The present submission has indicated that for all rotational plant commodities examined, TRR is greater than 0.01 ppm, and even if the tolerance expression were limited to parent and chloro metabolites, combined residues were greater than 0.02 ppm for mature spinach leaves, mature beet tops, and wheat forage, even at the maximum plantback intervals used.

Recommendation: In accordance with Conclusions 2c and 2d above, the present submission satisfies reregistration data requirements for Guideline 165-1, Confined Rotational Crops, and Guideline 165-2, Field Rotational Crops, Limited Trials. Depending on the tolerance expression and the limits of quantitation of the enforcement method, it is expected that extensive field trials will be required to support reregistration for rotational crops. CBRS advises that a more definitive determination of the tolerance expression, and therefore of requirements for

rotational crop field trials, should be available after review of the hydroxyatrazine cancer study. Field trials should be conducted with non-radioactive test substance, but conservative assumptions will be used to estimate total triazine ring residues for exposure assessment. We also advise that dietary exposure to atrazine residues is likely to be increased by residues on foods from rotational crops.

In its present submission, registrant noted that data on wheat as a rotational crop were relevant to registered use on fallow fields to be planted in wheat. CBRS has previously determined anticipated residues for combined parent and chloro metabolites from field trial data; anticipated residues for fallow use on wheat were 0.43 ppm for forage, 0.10 ppm for straw, and 0.02 ppm for grain (DEB 3688-3703, 9/14/88, M.S. Metzger). These anticipated residues were based on PHIs of 365 days for forage, and 650 days for straw and grain.

In a subsequent review of registrant's 90 day response to the DCI of 10/90, CBRS had the following comments on data requirements for wheat (CBRS 9167, 1/22/92, M.S. Metzger):

Metabolism studies are not available which would allow estimation of total triazine ring residues from wheat fallow applications. Detectable residues of parent and chlorometabolites were found in forage, straw, and grain at PHIs greater than 1 year. Since metabolism studies in corn and sorghum indicate that the percentage of the total residue accounted for by parent and chlorometabolites decreases as PHI increases, the presence of detectable residues of parent and chlorometabolites at such long PHIs in wheat suggests that the total residues of all components with a triazine ring may be considerably higher than residues of parent alone. Therefore, field metabolism studies for wheat are required.

In the present submission, maximum combined residues of parent and chloro metabolites in wheat commodities were 0.23 ppm in forage, 0.10 ppm in mature straw, and less than 0.01 ppm for mature grain; these data were from rotational crops with a maximum PHI of 13 months. These levels are comparable to (wheat straw) or less than anticipated residues determined from field trial data for fallow use, at PHIs up to 650 days. As CBRS has already noted (Ibid.), because of longer PHIs, metabolism may be more extensive for wheat fallow use than rotational crop use, and the ratio of total triazine ring:parent and chloro residues could be higher for wheat fallow use. These observations lead to the following conclusion:

Conclusion 3: The present submission is considered relevant to the early metabolic pathway of atrazine from wheat fallow application, but is inadequate to determine the magnitude of total triazine ring residues resulting from such use.

Recommendation: In accordance with Conclusion 3, the requirements of the 10/90 DCI for data on wheat fallow use have not been met. Data are still required on wheat fallow application that would at least indicate a ratio between combined residues of parent and chloro metabolites:total triazine ring residues. These data could best be provided by a radiolabel study that determines TRR and residues of parent and chloro metabolites.

Analytical Methods

To support the development of analytical methodology capable of measuring atrazine metabolites, and to validate those methods using radiolabeled samples from metabolism studies, registrant Ciba-Geigy provided the following document with the present submission:

Validation of Analytical Method AG-596 for the Determination of G-34048 and GS-17794 in Sugarcane and Wheat: Determination of the Radioactive Accountability of Hydroxy- and Chloro-Metabolites of Atrazine in ^{14}C-Atrazine Treated Crops by Analytical Methods AG-596 and AG-484, Including Residue Data, Ciba-Geigy Corporation, Project ABR-93053, October 26, 1993 (MRID 43016504).

Method AG-484 detects parent atrazine and chloro metabolites G-30033, G-28279, and G-28273; Method AG-596 detects the hydroxy metabolites G-34048 and GS-17794 (for structures, see Figure 1). With Method AG-484, a crop sample is refluxed in aqueous methanol, then cooled and filtered. An aliquot of extract is concentrated, then diluted with water and loaded onto an Extrelute column. The sample on the column is eluted with two different ratios of hexane:ethyl acetate to produce fractions A and B. Fraction A, containing atrazine, G-30033, and G-28279, is cleaned up on an Alumina B SepPak before GC analysis. Fraction B, containing G-28273, is cleaned up on a Florisil SepPak before GC analysis. The two fractions are analyzed separately by GC using DB-WAX columns.

With Method AG-596, a crop sample is refluxed in aqueous methanol, then cooled and partitioned with dichloromethane:hexane (80:20) in order to remove parent and chloro metabolite compounds. The aqueous extract and solids are diluted with 0.5 N HCl in methanol, refluxed again, cooled, and filtered. An aliquot of the extract is cleaned up by Sephadex G-10 gel chromatography, and then by a cation exchange column. The eluate is concentrated for analysis by HPLC with UV detection. HPLC analysis of GS-17794 and G-34048 is conducted on a C8 column switched to an SCX column, with separate mobile phase systems used for each compound. The detected compounds migrate as individual peaks; residues are quantitated based on peak heights compared with those of standards.

The Registrant validated each method using control samples of sugarcane and wheat, fortified with each of the residues to be detected. Control samples were sugarcane leaves and cane, wheat forage from IL, and wheat grain and straw from NY, since samples of these treated commodities were used for radiovalidation. Table 14 summarizes recovery data for Method AG-484, and Table 15 summarizes recovery data for Method AG-596, at the lowest fortification levels examined with each method:

Table 14. Recoveries from Fortified Samples with Method AG-484.

Sample, Fortification in ppm	% Recovery for residue:			
	Atrazine	G-30033	G-28279	G-28273
Sugarcane leaves, 0.05	95	78	78	66
Sugarcane cane, 0.05	101	103	98	81
Wheat forage, 0.05	100	115	94	81
Wheat straw, 0.05	69	70	58	65
Wheat Grain, 0.01	101	92	91	87

Table 15. Recoveries from Fortified Samples with Method AG-596

Sample, Fortification in ppm	% Recovery for metabolite:	
	G-34048	GS-17794
Sugarcane leaves, 0.02	70	106
Sugarcane cane, 0.02	102	113
Wheat forage, 0.02	82	94
Wheat straw, 0.02	95	104
Wheat Grain, 0.025	84	101

The Registrant also examined the ability of Methods AG-484 and AG-596 to detect residues from radiolabled samples from the corn and sorghum metabolism studies. Table 16 summarizes results for Method AG-484, and Table 17 for Method AG-596.

Table 16. Residues detected by Method AG-484 v. residues expected from metabolism studies.

Commodity	Atrazine, ppm:		G-30033, ppm:		G-28279, ppm:		G-28273, ppm:	
	Detected	Expected	Detected	Expected	Detected	Expected	Detected	Expected
Sugarcane leaves	≤0.05	0.017	≤0.05	0.027	≤0.05	0.015	0.22	0.426
Sugarcane cane	≤0.05	0.007	≤0.05	0.029	≤0.05	0.009	≤0.05	0.063
IL Wheat 60 DAP forage	0.128	0.142	0.071	0.072	≤0.05	0.007	≤0.05	0.012
NY Wheat straw	≤0.05	0.005	≤0.05	0.019	≤0.05	0.002	≤0.05	0.072
NY Wheat grain	≤0.01	<0.001	≤0.01	<0.001	≤0.01	<0.001	≤0.01	0.006

Table notes: Values for residues detected represent the average of three determinations. Expected values are based on Tables 5 and 11.

For both of Method AG-484 and AG-596, the plant sample was extracted with aqueous methanol, which was also used for the initial extraction of sugarcane and rotational crops by Extraction Method I in the present submission (see Figure 2). Sugarcane cane was extracted with the extended version of Method I, under which the initial unextracted residue received further treatment. The extended protocol for Extraction Method I would be expected to release conjugated residues, while Method AG-596 would be expected to extract free hydroxy residues. Therefore, in Table 17, results from Method AG-596 are compared with results from radiolabeled samples extracted by the initial steps of Method I. This consideration is not important in comparisons with Method AG-484, since the extended protocol did not release additional detectable residues of parent or chloro metabolites; nor with NY wheat grain, because even with the extended protocol, residues measured by Method AG-484 and AG-596 were still below the limits of determination.

Table 17. Residues detected by method AG-596 v. residues expected from metabolism studies.

	G-34048, ppm:		GS-17794, ppm:	
Sample	Detected	Expected	Detected	Expected
Sugarcane leaves	0.36	0.076	1.09	1.35
Sugarcane cane	≤0.02	<0.001	0.085	0.116
IL Wheat 60 DAP Forage	0.021	0.002	0.024	0.010
NY Wheat straw	≤0.02	0.001	≤0.02	0.009
NY Wheat grain	0.041, ≤0.02, ≤0.02	<0.001	0.043, ≤0.02, ≤0.02	0.001

Table notes: Values for residues detected represent the average of three determinations, except for wheat grain, where the detectable residues are believed to be due to contamination. Expected values are based on Table 11 for wheat, Table 5 for sugarcane leaves, and Table IX of MRID 430165035 for sugarcane cane. Metabolism results, reported in atrazine equivalents, were converted to values for the applicable metabolite.

CBRS Comments, Analytical Methods

Considering that they were obtained at the limits of determination, the recoveries from fortified samples in Tables 14 and 15 represent acceptable ranges.

Conclusion 4: Conclusions under this heading apply to analytical method.

Conclusion 4a: Recoveries from fortified samples were adequate for Methods AG-484 and AG-596. The limits of determination for Method AG-484 were the lowest levels analyzed, 0.05 ppm in sugarcane leaves and cane and in wheat forage and straw, and 0.01 ppm in wheat grain, for each of parent atrazine and chloro metabolites G-30033, G-28279, and G-28273. The limits of determination for Method AG-596 were the lowest levels analyzed, 0.025 ppm in wheat grain and 0.02 ppm in sugarcane leaves and cane and in wheat forage and straw, for each of the hydroxy metabolites G-34048 and GS-17794.

With regard to validation of Method AG-484 with samples from the metabolism studies, Table 16 indicates that in most cases, the levels of individual residues were below the limits of detection for the method. In wheat forage, however, there was good agreement between expected and detected residue levels of parent and G-30033. With metabolite G-28273, levels detected in sugarcane leaves were about half of those expected, and residues were undetectable in sugarcane cane and wheat straw, when they would have been expected to be above the method limit of determination. The Registrant noted that, in the metabolism studies, residues of G-28273 were assigned based on the profile from Aminex A-4 chromatography; the peak for this compound elutes early in a region where other polar compounds may be present. The Registrant therefore claimed that the assignment from the metabolism study may overestimate levels of G-28273. Even without this consideration, radiovalidation indicates that detection of this metabolite at levels of approximately 0.4 ppm is acceptable.

Conclusion 4b: Radiovalidation data for Method AG-484 were sufficient to validate the ability of the method to detect residues of parent atrazine and G-30033 near the limits of determination in wheat forage, and residues of G-28273 near 0.4 ppm. For G-28279, radiovalidation confirmed the limits of determination in sugarcane and wheat samples.

Table 17 indicates good agreement between detected and expected levels of GS-17794 in sugarcane samples, where detectable residues were expected. For G-34048, however, radiovalidation was not successful: detected levels in sugarcane leaves were much higher than expected, and the method detected residues in wheat forage, where detection would not have been expected.

Conclusion 4c: Radiovalidation data for Method AG-596 were sufficient to validate the ability of the method to detect residues of GS-17794 at levels of 0.12 ppm or higher. This method is not considered radiovalidated for compound G-34048.

cc:Circ, Abbotts, RF, Atrazine List A File, SF
RDI:FBSuhre:6/15/95:RBPerfetti:6/21/95:EZager:6/28/95
H7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:6/28/95
JA12\atrazine.8