6-3-93

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

JUN 3 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Atrazine, Reregistration and Special Review.
Registrant Ciba-Geigy Submission of Overview (MRID 42547115) and Data on Metabolism in Corn and Sorghum (MRID 42547116), Magnitude of the Residue in Corn (MRID 42547117), and Analytical Methods (MRIDs 42547118 through 42547123)
CBRS No. 10980. DP Barcode No. D185491.

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In response to previous reviews and a reregistration Data Call-In of 10/90, registrant Ciba-Geigy Corporation has submitted additional data on the nature of the residue in corn and sorghum, magnitude of the residue in corn, and analytical methods. These data were part of a larger submission and are described in a cover letter of November 2, 1992 from Ciba-Geigy to SRRD. Assignment instructions are to review MRID Nos. 42547115 through 42547123 to determine if they fulfill residue chemistry guideline requirements. The conclusions below pertain only to the data submitted.

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.

Conclusions

1a. CBRS reiterates that if the registrant wishes to maintain registrations on sweet corn, field metabolism studies for sweet corn are required.

1b. For the purposes of these field radiolabel studies, studies with post-emergence application to corn in IL, MS, and NY are considered representative geographically, of soil types, and of registered uses.

1c. The protocols for the field corn metabolism studies should be provided.

1d. Registrant should describe storage conditions for corn samples, including times and approximate temperatures of storage, between harvest and shipment to the performing laboratory.

1e. No data for corn were provided which alter the position of the HED Metabolism Committee that exposure assessment for atrazine should be conducted on the basis of the total radioactive residue (Memo, 8/7/92, M.S. Metzger).

1f. If storage conditions were adequate (Conclusion 1d), averaging residues from the present studies with those from a previous corn metabolism study in the field would result in a modest reduction in anticipated residues for corn commodities. Revised anticipated residues for corn and sorghum will be provided in a separate memorandum.

1g. The present submission indicates variability in the composition of the total residue in corn with location and commodity, and variability in residues detected with method of extraction. In the present studies, the percent of TRR represented by organic soluble residues ranged from 0.5% to 3.7% in grain and from 2.6% to 49.8% in forage and fodder; the percent of TRR assigned to known hydroxy metabolites ranged from 3.9% to 16.6% in grain, and from 13.6% to 40% in forage and fodder. If a

marker method is ultimately accepted, conservative assumptions will be necessary for determining appropriate tolerance levels.

1h. The present submission identified in corn an organic soluble unknown which may be a chloro triazine, and indicated that mild acid hydrolysis can release chloro triazine residues. In the absence of additional data on the identity of metabolites, organic soluble residues extracted under mildly acidic conditions should be assumed to represent chloro triazine compounds.

1i. The assignment of residues as known hydroxy metabolites was confirmed by a second analytical method only for residues extracted using methanol:water (Extraction Method I). In the absence of additional data on the identity of metabolites extracted under mildly acidic conditions, extraction by methanol:water should be assumed to provide a more reliable assignment of the known hydroxy metabolites.

2a. For the purposes of these field radiolabel studies, studies with post-emergence application to sorghum in IL, MS, and NY are considered representative geographically, of soil types, and of registered uses.

2b. The protocols for the sorghum metabolism studies should be provided.

2c. Registrant should describe storage conditions for sorghum samples, including times and approximate temperatures of storage, between harvest and shipment to the performing laboratory.

2d. No data for sorghum were provided which alter the position of the HED Metabolism Committee that exposure assessment for atrazine should be conducted on the basis of the total radioactive residue (Memo, 8/7/92, M.S. Metzger).

2e. Averaging residues from the present studies with those from previous studies would not be expected to produce a significant change in anticipated residues for sorghum commodities. Revised anticipated residues for corn and sorghum will be provided in a separate memorandum.

2f. The present submission indicates variability in the composition of the total residue in sorghum with location and commodity, and variability in residues detected with the method of extraction. In the present studies, the percent of TRR represented by organic soluble residues ranged from 1.0% to 4.3% in sorghum grain and from 4.3% to 54.9% in sorghum forage and fodder; the percent of TRR represented by known hydroxy metabolites ranged from 2.9% to 17.4% in sorghum grain and from 5.3% to 28.8% in sorghum forage and fodder. If a marker method is ultimately acepted, conservative assumptions will be necessary for determining appropriate tolerance levels.

2g. The present submission identified in sorghum organic soluble unknowns which may be chloro triazines, and the ability of mild acid hydrolysis to release chloro triazines from unextracted residues was not reported. In the absence of additional data on the identity of metabolites, organic soluble residues extracted under mildly acidic conditions should be assumed to represent chloro triazine compounds.

2h. The present submission found that if sorghum samples were extracted under mildly acidic conditions, aqueous soluble fractions contained residues different from the known hydroxy metabolites, but which showed similar chromatographic mobility as these hydroxy metabolites. In the absence of additional data on the identity of metabolites extracted under mildly acidic conditions, extraction by methanol:water (Extraction Method I) should be assumed to provide a more reliable assignment of the known hydroxy metabolites.

3a. 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, 0.001 ppm in corn grain, 0.01 ppm in sorghum grain, and 0.05 ppm in other corn and sorghum commodities, for each of parent atrazine, G-30033, G-28279, and G-28273. The limits of determination for Method AG-596 were the lowest levels analyzed, 0.02 ppm in corn and sorghum commodities, for G-34048 and GS-17794.

3b. Radiovalidation data for Method AG-484 are sufficient to validate the ability of the method to detect residues of parent atrazine in the range of 1-2 ppm.

3c. Radiovalidation data for Method AG-596 are sufficient to validate the method's ability to measure residues of compound GS-17794 at levels greater than 0.02 ppm in corn and sorghum.

3d. Method AG-596 uses mild acid hydrolysis, which releases compounds that could artificially inflate detected residues of the hydroxy metabolites (see, for example, Conclusion 2h). Because there has not been a precise determination of the known hydroxy metabolites released from metabolism samples by mild acid hydrolysis, Method AG-596 is not considered radio-validated for compound G-34048.

4a. CBRS acknowledges that the requirement for methodology to measure hydroxytriazine residues in animal commodities (Guideline 171-4(d)) is reserved, pending review of the hydroxyatrazine chronic feeding/oncogenicity study.

4b. CBRS has previously declined review of data on independent laboratory validation of Methods AG-484 (MRID 41397102) and

AG-476 (MRID 41397103) and on multiresidue protocols (MRID 41423401), because data were not provided for the total residue of concern.

4c. Validation by registrant and independent laboratory validation of Method AG-496A using fortified samples were acceptable. The limit of determination is 0.01 ppm, the lowest level analyzed, for each of parent atrazine, G-30033, G-28273, and G-28229. The reregistration data requirement for independent laboratory validation of Methods AG-436 and AG-496 (both replaced by Method AG-496A) for milk is fulfilled. CBRS defers referral of this method for validation by an Agency laboratory until results of the hydroxyatrazine carcinogenicity study are reviewed and accepted.

4d. Validation by registrant and independent laboratory validation of Method AG-593 using fortified samples were acceptable. The limit of determination is 0.01 ppm, the lowest level analyzed, for each of parent atrazine, G-30033, G-28273, and G-28229. The reregistration data requirement for independent laboratory validation of Method EN-CAS 86-284 (replaced by Method AG-593) using eggs and poultry tissues is fulfilled. CBRS defers referral of this method for validation by an Agency laboratory until results of the hydroxyatrazine carcinogenicity study are reviewed and accepted.

5. With non-radiolabeled field trials in the midwest, residues of six marker compounds in or on corn grain were nondetectable at application rates of 1X. The combined limit of detection for the marker compounds was 0.08 ppm for corn grain. The residue data provided do not alter the conclusion that exposure assessment should be conducted on the basis of the total ^{14}C-labeled residue (Conclusion 1e). In addition, storage stability studies to support data on two of the marker compounds are in progress. CBRS declines review of the magnitude of the residue studies in corn, until results of the hydroxyatrazine carcinogenicity study are reviewed and accepted.

Recommendations

Before the data submitted from field metabolism studies of field corn and sorghum can be accepted, the registrant must resolve the deficiencies identified in Conclusions 1c, 1d, 2b, and 2c. As indicated in Conclusions 1e and 2d, exposure assessment in corn and sorghum should continue to be conducted on the basis of the total radioactive residue.

CBRS acknowledges that the present submission represents considerable scientific effort, and the work appears to be competently done. However, CBRS must defer a conclusion on the marker method. The HED Metabolism Committee has concluded that if data indicating that hydroxyatrazine is not carcinogenic were

reviewed and accepted, then exposure assessment and tolerance expression for atrazine would include only parent and chloro metabolites. If such an alteration in the residues of concern were to occur, then it would seem feasible that methods presently available could be developed for tolerance enforcement. CBRS therefore reserves judgement on the proposed marker methods, pending review of data on the carcinogenicity of hydroxyatrazine.

In accordance with Conclusions 4c and 4d, the present submission fulfilled some reregistration requirements for analytical methods.

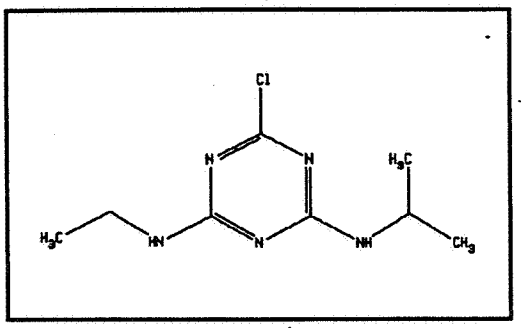
DETAILED CONSIDERATIONS

NATURE OF THE RESIDUE

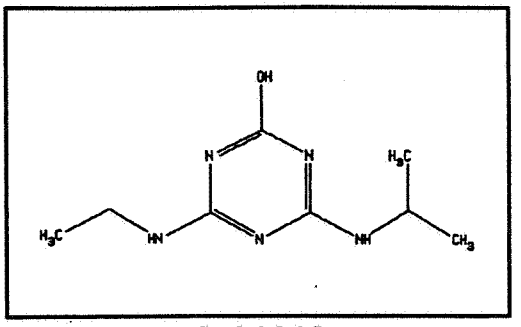
Reregistration Requirements

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.

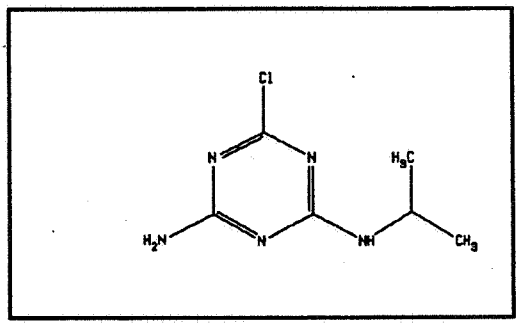
A subsequent review (DEB No. 5783, 5/3/90, M.S. Metzger) noted that all metabolites containing the intact triazine ring were now considered of toxicological concern, and data requirements should be revised such that all metabolites which contain the intact triazine ring are determined for all commodities 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).



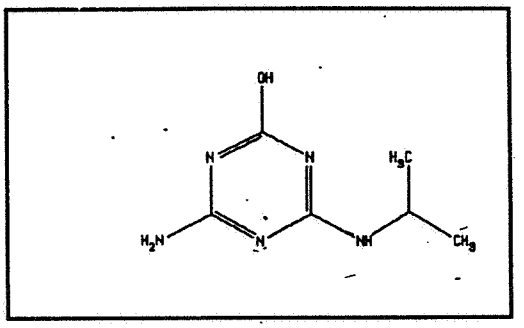
Atrazine



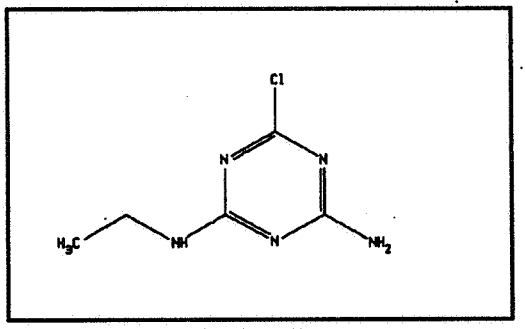
G-34048



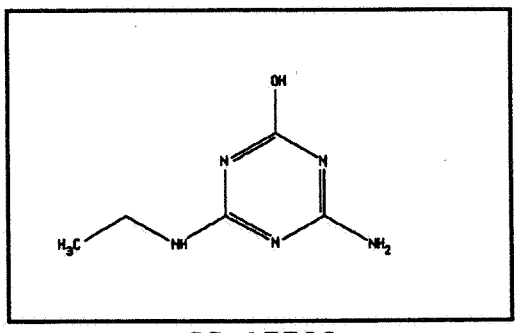
G-30033



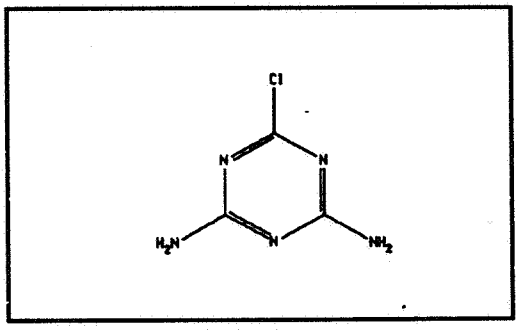
GS-17794



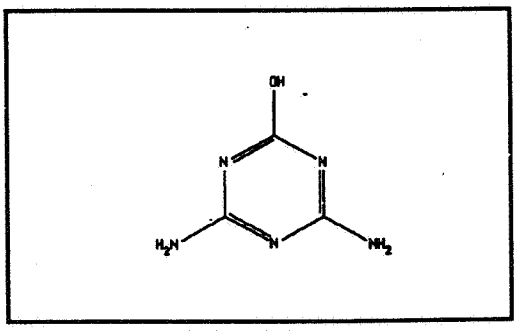
G-28279



GS-17792



G-28273



GS-17791

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

The HED Metabolism Committee addressed issues related to triazine chemicals at a meeting of 7/9/92 (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 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, 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.

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

^{14}C-Atrazine: Nature of the Residue in Corn and Sorghum, Hazelton Wisconsin, Inc., Project HWI 6117-178, October 26, 1992 (MRID 42547116).

Registrant also submitted a document which summarized data on nature and magnitude of residues in corn and sorghum:

Atrazine - Overview of the Nature and Magnitude of Potential "Marker" Metabolites in Corn and Sorghum. Analysis of Field Samples Treated with ^{14}C-Atrazine or Aatrex Nine-O, Ciba-Geigy Corporation, Project ABR-92068, October 28, 1992 (MRID 42547115).

NATURE OF THE RESIDUE, CORN

Field Procedures

Plots 6 x 14 ft were prepared for field corn at Ciba-Geigy research stations in MS (silty loam soil), IL (clay loam), and NY (loam). One half of each plot was planted with corn, and the other half with sorghum. Field corn seed (a different variety at each plot) was planted in three rows at each plot. 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. In its Overview document (MRID 42547115), registrant notes that the maximum use rate on corn and sorghum has been reduced to 2.5 lb ai/A. The test substance was applied in MS at a corn height of 8 in, in IL at a corn height of 10-12 in, and in NY at a corn height of 12-14 in. Control plots at each location were treated in the same manner, but with unlabeled atrazine. Corn leaves were sampled on the day of application; corn forage samples were collected at 30 days and at silage stage (46-75 days), and corn grain and fodder were harvested at maturity (85-106 days). At maturity, ears were removed from the stalk, husks were removed from ears and discarded, and stalks and leaves were collected as fodder samples. Control samples were harvested and processed in the same fashion as treated samples. Samples were collected by hand, placed into prelabeled plastic bags, and stored in a freezer. 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.

CBRS Comments, Corn Field Procedures

In the previous review of Ciba-Geigy's response to the DCI of October 1990, CBRS noted that if the registrant wishes to maintain registrations on sweet corn, field metabolism studies for sweet corn must be submitted (CBRS No. 9167, 1/22/92, M.S. Metzger). The registrant's description of field procedures indicated that only field corn was planted at each of the three locations.

Conclusion 1a: CBRS reiterates that if the registrant wishes to maintain registrations on sweet corn, field metabolism studies for sweet corn are required.

The previous review (CBRS No. 9167) concluded that corn and sorghum can be treated both pre- and post-emergent, and both the residue profile and magnitude will vary depending on the time of application. CBRS recommended a discussion of how the proposed studies will supplement available studies to provide data representative geographically, of soil types, and of registered uses. In response, registrant noted in its Overview document (MRID 42547115) that most of the previous metabolism studies have been conducted with pre-emergence application, and in these studies hydroxy metabolites represented the predominant residues. Registrant noted that post-emergence treatment was expected to result in higher total radioactive residues, and in a more

complex mixture of metabolites, because of the potential for uptake of chloro triazines into treated leaves. In support of its comments, registrant referred to a previously submitted overview on atrazine metabolism, Report ABR-89060 (MRID 41209801). This previous report has already been reviewed (DEB No. 5783, 5/3/90, M.S. Metzger), and the review supports the claim that polar metabolites predominate with pre-emergence application.

With regard to the matter of geographic diversity with the field metabolism studies, registrant noted that each location represented a different soil type. In addition, the 10/90 DCI noted that for reregistration, magnitude of the residue studies would eventually be required in IL (17%) or IA (19%), IN (9%) or OH (6%), MN (8%) or WI (4%), and NE (11%), which collectively represent greater than 70% of 1985 U.S. field corn production. For the purposes of geographic representation of field radiolabel studies only, the study in IL can be considered representative of midwestern states. The studies in NY and MS were outside the major corn growing areas of the U.S., and can be considered appropriate for diversity of field metabolism studies.

Conclusion 1b: For the purposes of these field radiolabel studies, studies with post-emergence application to corn in IL, MS, and NY are considered representative geographically, of soil types, and of registered uses.

In the previous review (CBRS No. 9167), CBRS recommended that a detailed protocol be submitted for review prior to initiation of these studies. The present submission (MRID 42547116) stated that the protocol was provided as Appendix A. However, Appendix A contains a single page listing deviations from the protocol. The protocol was not provided.

Conclusion 1c: The protocols for the field corn metabolism studies should be provided.

While frozen storage conditions were described for storage at the performing laboratory, storage conditions between harvest and storage at the field site, and prior to shipment to the performing laboratory, are not immediately obvious. These data should be provided.

Conclusion 1d: Registrant should describe storage conditions for corn samples, including times and approximate temperatures of storage, between harvest and shipment to the performing laboratory.

Laboratory Analysis

Total radioactive residues (TRR) of corn samples were determined by combustion and liquid scintillation counting (LSC). Results

are summarized in Table 1; forage, silage, and fodder samples are described above under Field Procedures. Corn leaf samples collected at day 0 after application were also analyzed; TRR in these samples was described as 204 to 557 ppm, but values at each location were not provided.

Table 1. TRR in Corn Samples.

Location	Sample	Days after Treatment	TRR, ppm
MS	Corn Forage	30	0.694
	Corn Silage	75	0.660
	Corn Fodder	98	0.850
	Corn Grain	98	0.045
IL	Corn Forage	30	0.466
	Corn Silage	46	0.710
	Corn Fodder	85	1.809
	Corn Grain	85	0.071
NY	Corn Forage	30	2.84
	Corn Silage	72	0.499
	Corn Fodder	106	1.549
	Corn Grain	106	0.034

Radioactive residues in corn samples were extracted by two similar methods. Method II is shown in Figure 2. In Method II, samples were extracted by refluxing with 0.5 N HCl in methanol for approximately 1.5 h. The reflux mixtures were filtered and rinsed with methanol. Filtrate and rinses were collected as one sample and methanol was removed by evaporation. The sample was redissolved in water and neutralized with ammonium hydroxide, and then partitioned with equal volumes of chloroform. The chloroform layers were combined and partitioned once with an equal volume of water. The water layers were combined with the aqueous layer from the extract. Radioactivity in aqueous and organic fractions was determined by liquid scintillation counting.

Extraction Method I was similar. Samples were extracted in 20 to 40 g batches with 50 to 100 ml of methanol:water (80:20, v:v). Tissues were ground in an ice bath for approximately 20 min each. The extract was centrifuged and the supernatant fraction was decanted. This procedure was repeated with the nonextracted residue fraction until radioactivity in the supernatant fraction was negligible. Supernatant fractions were pooled.

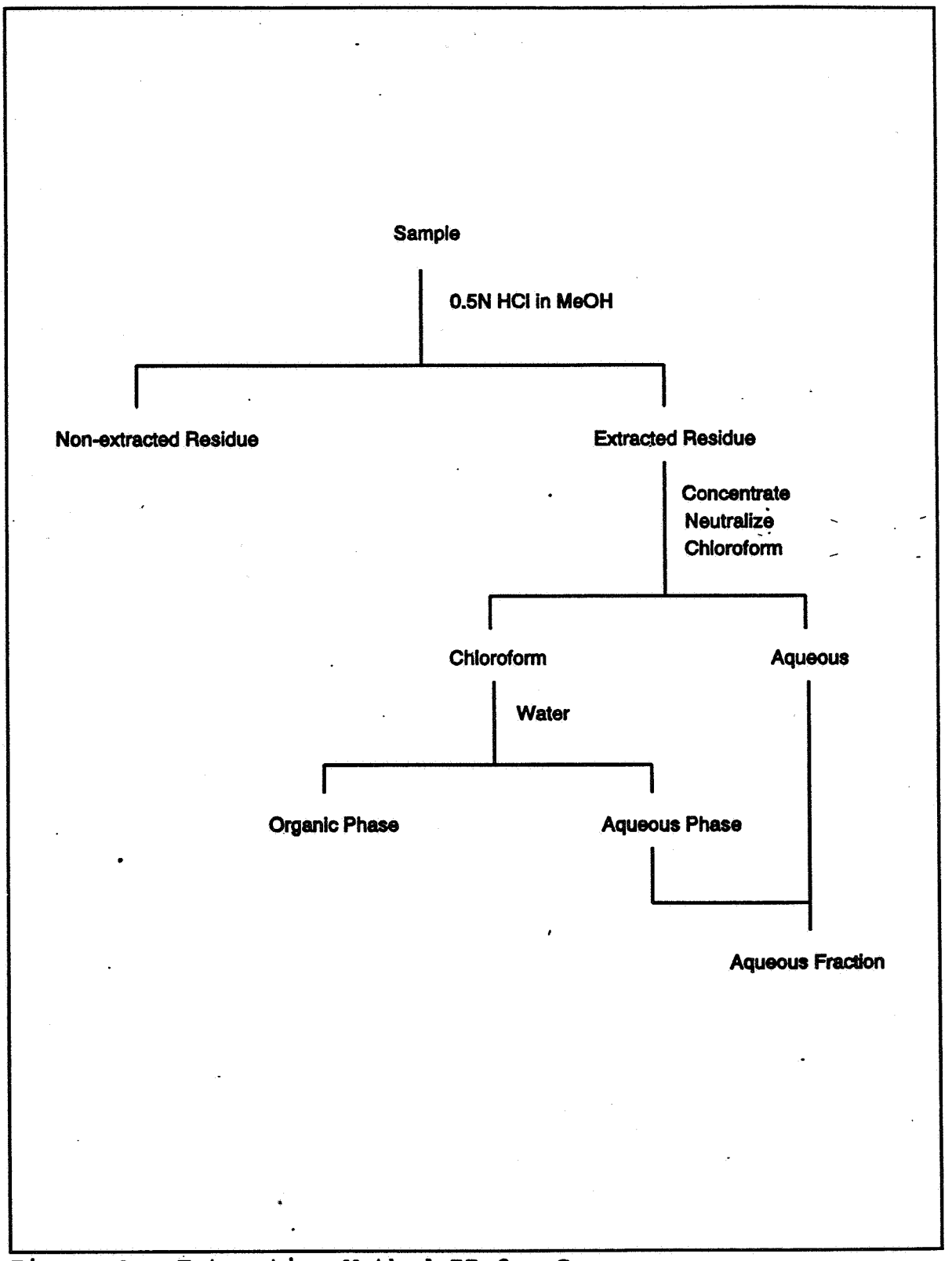


Figure 2. Extraction Method II for Corn

Methanol was evaporated, and the aqueous solution was partitioned three times with equal volumes of chloroform. Chloroform layers were pooled, and the resulting chloroform fraction was partitioned once with an equal volume of water. The two aqueous fractions were combined as with Method II.

Nonextractable residues and aqueous extract fractions were further treated by acid hydrolysis by refluxing with HCl at different concentrations for 4 h. The hydrolysis mixtures were filtered and rinsed with methanol. The filtrate and rinses were combined, neutralized with ammonium hydroxide, and partitioned with chloroform or ethyl acetate. In addition, the nonextracted residues from the 30 day IL corn sample were extracted by Soxhlet with chloroform for 4 h and with methanol for 8 h to further extract radioactivity.

Extraction Method I was used for all corn samples from these studies; Method II was used for selected samples. The distributions of TRR with these methods are summarized in Table 2:

Table 2. Extraction of TRR from Corn Samples.

Sample		TRR, ppm	% TRR Extracted by Method I as:			
			Organic	Aqueous	Non-extracted	Total
MS	30 Day Forage	0.694	9.2	61.5	27.1	97.8
	Silage Forage	0.660	5.2	58.7	30.8	94.7
	Mature Fodder	0.850	2.6	49.6	40.7	92.9
	Mature Grain	0.045	0.5	41.4	54.3	96.2
IL	30 Day Forage	0.466	8.4	61.7	24.0	94.1
	Silage Forage	0.710	14.6	65.4	26.9	106.9
	Mature Fodder	1.809	5.4	52.9	39.3	97.6
	Mature Grain	0.071	1.5	36.7	60.2	98.4
NY	30 Day Forage	2.840	49.8	36.5	13.6	99.9
	Silage Forage	0.499	9.2	61.5	19.4	90.1
	Mature Fodder	1.549	9.1	53.4	39.4	101.9
	Mature Grain	0.034	1.0	38.8	52.4	92.2
Sample		TRR, ppm	% TRR Extracted by Method II as:			
			Organic	Aqueous	Non-extracted	Total
MS	30 Day Forage	0.694	8.2	67.7	17.9	93.8
	Mature Fodder	0.850	5.6	64.0	26.9	96.5
IL	30 Day Forage	0.466	15.7	59.1	16.4	91.2
	Mature Fodder	1.809	10.6	56.2	23.6	90.4
NY	30 Day Forage	2.840	30.1	57.4	13.2	100.7
	Mature Fodder	1.549	15.2	66.9	21.7	103.8
	Mature Grain	0.034	3.7	52.3	51.4	107.4

Table note: Not all samples were extracted by Method II.

Residue Identification

Fractions extracted by Method I were further analyzed to identify residues. Organic fractions were analyzed by 2-dimensional thin layer chromatography (2-D TLC) using silica gel plates and two different combinations of solvent systems. In TLC Method I, 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). In TLC Method II, the solvent in the first dimension was ethyl acetate:hexane (30:70), and the solvent in the second dimension, for two developments, was toluene:acetone (75:25). Aqueous fractions were analyzed by Aminex A-4 cation exchange chromatography. Column buffer was 0.1 M ammonium formate, pH 4. After application of sample, the column was washed in this buffer, then eluted with a linear gradient to 1.0 M ammonium formate, pH 6, followed by 0.1 M ammonium hydroxide.

Peaks from TLC and Aminex chromatography were assigned based on similar mobilities with standards atrazine and its chloro and hydroxy metabolites indicated in Figure 1. Atrazine and chloro metabolites were identified in organic fractions. Hydroxy metabolites were chiefly identified in aqueous fractions, and to a lesser extent in the organic fractions. Organic fractions in grain were not characterized, on the grounds that total radioactivity was low. The assignment of residues from Extraction Method I is summarized in Table 3; the assignment of residues in the organic fractions only is summarized in Table 3A.

A significant residue in most of the organic soluble fractions was Unknown 1, which showed a mobility on 2-D TLC intermediate between the chloro metabolites G-28279 and G-28273. Registrant could not rule out the possibility that Unknown 1 was a chlorotriazine. Analysis of aqueous fractions by Aminex chromatography generally revealed at least 10 to 12 components. Peaks were identified corresponding to standards for the atrazine hydroxy metabolites indicated in Figure 1, and identification of these peaks as the hydroxy metabolites was confirmed by HPLC analysis. Metabolite GS-17794 represented the largest single metabolite in most of the corn samples. Peaks No. 1 through 9 were also identified as components of the aqueous fraction, and reference to the published literature suggested that these residues may be conjugates of atrazine and dealkylated triazines. However, these peaks were not further analyzed. With the exception of Peak No. 1, each of these peaks generally represented less than 10% of TRR; in addition, Peak No. 1 was subsequently shown by DEAE Sephadex A-25 anion exchange chromatography to consist of more than one component.

Fractions obtained from Extraction Method II were also analyzed further. Although Method II in most cases increased the % TRR extracted in organic fractions, these fractions were not further characterized. Aqueous fractions were characterized and hydroxy metabolites were identified as described for Extraction Method I. Method II resulted in increased assignment of aqueous soluble residues as the hydroxy metabolites, presumably by the release of some conjugated residues. Table 4 summarizes a comparison between Methods I and II of residues assigned as hydroxy metabolites. The performing laboratory noted that because

Table 3. Assignment of Residues in Corn, Extracted by Method I.

Residue	Percent of TRR assigned by location and sample:											
	MS				IL				NY			
	30-Day Forage	Silage Forage	Mature Fodder	Mature Grain	30-Day Forage	Silage Forage	Mature Fodder	Mature Grain	30-Day Forage	Silage Forage	Mature Fodder	Mature Grain
Organic Fraction	(9.2)	(5.2)	(2.6)	(0.5) NA	(8.4)	(14.6)	(5.4)	(1.5) NA	(49.8)	(9.2)	(9.1)	(1.0) NA
Atrazine	0.4	<0.1	<0.1		2.0	1.3	0.2		43.2	1.1	0.8	
G-28273	0.2	0.2	0.1		0.1	0.2	<0.1		0.1	0.1	<0.1	
G-28279	0.2	0.1	<0.1		0.3	0.2	0.1		0.2	0.1	0.2	
G-30033	0.3	0.1	<0.1		0.3	0.1	0.1		0.4	0.2	0.5	
Unknown 1	1.1	0.8	1.0		2.3	2.1	2.7		1.6	2.6	4.1	
Hydroxy:												
GS-17791	1.4	1.3	0.7	0.7	1.1	1.8	1.0	2.3	0.7	0.6	<0.1	1.2
GS-17792	4.4	4.5	3.3	0.5	4.1	5.2	4.5	1.3	1.1	2.0	1.7	0.9
GS-17794	18.5	19.8	14.6	2.7	16.8	19.9	16.9	6.1	8.1	18.6	14.4	10.7
G-34048	8.1	2.9	0.6	<0.1	6.1	9.1	3.0	<0.1	3.7	2.1	2.0	0.3
Total hydroxy:	32.4	28.5	19.2	3.9	28.1	36.0	25.4	9.7	13.6	23.3	18.1	13.1
Total, known compounds:	33.5	28.9	19.3	3.9	30.8	37.8	25.8	9.7	57.5	24.8	19.6	13.1

Table notes: See Figure 1 for structures. Assignments for hydroxy compounds represent combined residues in organic and aqueous phases. NA = organic fraction not analyzed.

Table 3A. Assignment of Residues in Corn, Organic Fractions from Method I.

Residue	Percent of TRR assigned by location and sample:								
	MS			IL			NY		
	30-Day Forage	Silage Forage	Mature Fodder	30-Day Forage	Silage Forage	Mature Fodder	30-Day Forage	Silage Forage	Mature Fodder
Total Organic Fraction	(9.2)	(5.2)	(2.6)	(8.4)	(14.6)	(5.4)	(49.8)	(9.2)	(9.1)
Atrazine	0.4	<0.1	<0.1	2.0	1.3	0.2	43.2	1.1	0.8
G-28273	0.2	0.2	0.1	0.1	0.2	<0.1	0.1	0.1	<0.1
G-23279	0.2	0.1	<0.1	0.3	0.2	0.1	0.2	0.1	0.2
G-30033	0.3	0.1	<0.1	0.3	0.1	0.1	0.4	0.2	0.5
Unknown 1	1.1	0.8	1.0	2.3	2.1	2.7	1.6	2.6	4.1
Hydroxy:									
GS-17792	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1
GS-17794	0.3	0.5	<0.1	<0.1	0.6	<0.1	<0.1	0.5	0.1
G-34048	5.1	2.3	0.1	2.0	6.7	0.7	1.3	2.1	0.9
Total hydroxy:	5.4	2.8	0.1	2.0	7.4	0.7	1.3	2.6	1.0
Total, known compounds:	6.5	3.2	0.2	4.7	9.2	1.1	45.2	4.1	2.5

Table notes: See Figure 1 for structures. Organic fractions from grains were not analyzed. GS-17791 was not detected in any organic fractions.

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Table 4. Comparison of Residue Assignment by Extraction Method I or Method II.

Residue	% of TRR in Corn Samples Assigned to:													
	MS				IL				NY					
	30-Day Forage		Mature Fodder		30-Day Forage		Mature Fodder		30-Day Forage		Mature Fodder		Mature Grain	
	Method I	II	I	II	I	II	I	II	I	II	I	II	I	II
Organic Fraction	9.2	8.2	2.6	5.6	8.4	15.7	5.4	10.6	49.8	30.1	9.1	15.2	1.0	3.7
Aqueous Fraction	61.5	67.7	49.6	64.0	61.7	59.1	52.9	56.2	36.5	57.4	53.4	66.9	38.8	52.3
Within Aqueous Fraction:														
GS-17791	1.4	2.6	0.7	5.7	1.1	1.6	1.0	3.0	0.7	1.1	ND	1.3	1.2	2.0
GS-17792	4.4	5.0	3.3	4.7	4.1	4.1	4.5	6.2	1.1	1.6	1.7	2.7	0.9	0.6
GS-17794	18.2	23.2	14.6	17.0	16.8	19.2	16.9	18.4	8.1	9.4	14.3	19.4	10.7	14.0
G-34048	3.0	9.2	0.5	2.1	4.1	7.3	2.3	4.0	2.4	24.2	1.1	2.3	0.3	ND
Total, Hydroxy in Aqueous	27.0	40.0	19.1	29.5	26.1	32.2	24.7	31.6	12.3	36.3	17.1	25.7	13.1	16.6

Table notes: ND = non-detectable

assignment of the hydroxy metabolites had been confirmed by a second analytical method with the use of Extraction Method I, assignment as hydroxy metabolites of residues obtained with Extraction Method II were not confirmed.

The nonextracted residue from NY fodder (37.5% TRR) was further treated by reflux in 0.1 N HCl, the extract was partitioned with chloroform, the organic phase was partitioned with water, and the aqueous phases were combined. This treatment released 2.6% TRR to the organic phase and 18.1% TRR to the aqueous phases. In the combined aqueous phases from this treatment, the known hydroxy metabolites were detected by Aminex chromatography, but each represented 1.5% TRR or less. A peak of radioactivity representing 10% TRR migrated with the same mobility as G-34048, but this peak was described as an unresolved mixture including other compounds. HPLC analysis of the organic phase from this reflux treatment indicated that 2.4% of TRR had the same mobility as G-28279. It should be noted that in the organic extract of NY fodder using Method I, G-28279 represented 0.2% TRR, and atrazine and known chloro metabolites combined represented 1.5% TRR (see Table 3); the implication is that the nonextracted residue may contain significant levels of chloro triazine compounds.

Aqueous fractions of corn fodder samples from IL and NY were incubated with ß-glucosidase. Little difference was observed in the profile from an Aminex A-4 column in extracts before or after treatment, indicating that glucose conjugates did not represent a significant portion of the total residue. Corn fodder samples from NY were extracted by Method I in November 1991, 2 mo after mature harvest, and in June 1992, 4 mo before the termination of laboratory analysis. Aminex A-4 chromatography profiles of the aqueous extracts were generally consistent, indicating adequate storage stability over the course of the study.

CBRS Comments, Residue Identification in Corn

This submission did not identify residues other than the chloro and hydroxy metabolites indicated in Figure 1. Therefore, no data were provided to indicate that the total radioactive residue may include metabolites other than those consisting of the triazine ring with a substituent. In the absence of additional data on the nature of the residue, the earlier conclusion that exposure assessment should be conducted on the basis of the total radioactive residue is not altered.

Conclusion 1e: No data for corn were provided which alter the position of the HED Metabolism Committee that exposure assessment for atrazine should be conducted on the basis of the total radioactive residue (Memo, 8/7/92, M.S. Metzger).

Anticipated residues for atrazine were previously determined from data obtained from metabolism studies in the greenhouse and in

the field, and included the following values (DEB No. 5873, 5/3/90, M.S. Metzger): Corn grain, 0.1 ppm; Corn forage, 5.0 ppm; Corn fodder and silage; 3.0 ppm. In the only previous field metabolism study where application was post-emergent, TRR was 5.4 ppm in stalks and 0.07 ppm in grain. In the present submission, the average residues across studies in MS, IL, and NY were 1.33 ppm in 30-day corn forage; 0.62 ppm in corn silage forage; 1.40 ppm in corn fodder, and 0.05 ppm in corn grain (see Table 1).

Conclusion 1f: If storage conditions were adequate (Conclusion 1d), averaging residues from the present studies with those from a previous corn metabolism study in the field would result in a modest reduction in anticipated residues for corn commodities. Revised anticipated residues for corn and sorghum will be provided in a separate memorandum.

Previous review noted that the composition of the residue in corn varies with crop and crop commodity (CBRS No. 9167, 1/22/92, M.S. Metzger): For example, the percent of TRR in corn represented by organic soluble residues varied from 0.5-22% in stalks and <0.1-21% in grain; and the percent represented by aqueous soluble residues varied from 27-70% in stalks and 47-78% in grain. Such variability was also encountered in the present submission, and field location and extraction method were indicated as additional factors contributing to variability. In the present studies, the percent of TRR represented by organic soluble residues ranged from 0.5% to 3.7% in grain and from 2.6% to 49.8% in forage and fodder; the percent of TRR assigned to known hydroxy metabolites ranged from 3.9% to 16.6% in grain, and from 13.6% to 40% in forage and fodder (see Tables 3 and 4). Previous review concluded that in cases where the entire residue containing the triazine ring was not measured, conservative assumptions will have to be used in conducting exposure assessment (CBRS No. 9167, Ibid.). The same considerations should apply if a marker method is ultimately accepted: conservative assumptions will have to be used for determining appropriate tolerance levels.

Conclusion 1g: The present submission indicates variability in the composition of the total residue in corn with location and commodity, and variability in residues detected with method of extraction. In the present studies, the percent of TRR represented by organic soluble residues ranged from 0.5% to 3.7% in grain and from 2.6% to 49.8% in forage and fodder; the percent of TRR assigned to known hydroxy metabolites ranged from 3.9% to 16.6% in grain, and from 13.6% to 40% in forage and fodder. If a marker method is ultimately accepted, conservative assumptions will be necessary for determining appropriate tolerance levels.

Residues of parent atrazine and the chloro metabolites in Figure 1 are known to be extractable into organic solvents (see,

for example, DEB No. 5783, 5/3/90, M.S. Metzger). The present submission identified Unknown 1 as a significant component of the organic soluble fraction (see Table 3), and registrant could not rule out the possibility that this compound was a chloro triazine. In some forage samples, hydroxy metabolites represented approximately half the residue in the organic fraction, but in other samples the contribution from the known hydroxy compounds was much smaller (see Table 3A). Conjugated residues have generally been expected to represent further metabolism of the hydroxy compounds. For example, the Second Round Review Residue Chemistry Chapter (10/18/88) did not report any conjugated metabolites which retain the Cl atom. In the present submission, however, treatment with 0.1 N HCl of unextracted residues from NY fodder released an entity with the same chromatographic mobility as the chloro metabolite G-28279, representing 2.4% of TRR. In contrast, atrazine and known chloro compounds in the original organic soluble fraction represented only 1.5% of TRR in this sample. The present submission thus indicates that Unknown 1 may be a chloro triazine, and chloro triazines may be present in the unextracted fraction. In the absence of additional data on the identity of metabolites, organic soluble residues extracted under mildly acidic conditions should be assumed to represent parent and chloro triazine metabolites.

Conclusion 1h: The present submission identified in corn an organic soluble unknown which may be a chloro triazine, and indicated that mild acid hydrolysis can release chloro triazine residues. In the absence of additional data on the identity of metabolites, organic soluble residues extracted under mildly acidic conditions should be assumed to represent chloro triazine compounds.

With regard to the hydroxy metabolites, the performing laboratory noted that treatment of sorghum samples by Extraction Method II, under mildly acidic conditions, could release compounds different from the known hydroxy metabolites, but which showed the same mobility during Aminex chromatography as the hydroxy metabolites (see Conclusion 2i and discussion under the section CBRS Comments, Residue Identification in Sorghum). The performing laboratory noted that caution should be used when interpreting the analytical results using Extraction Method II. Because the identities of the hydroxy metabolites obtained from Extraction Method I were confirmed by a second analytical method, the assignment of hydroxy metabolites under this method should be assumed to be more reliable.

Conclusion 1i: The assignment of residues as known hydroxy metabolites was confirmed by a second analytical method only for residues extracted using methanol:water (Extraction Method I). In the absence of additional data on the identity of metabolites extracted under mildly acidic conditions, extraction by

methanol:water should be assumed to provide a more reliable assignment of the known hydroxy metabolites.

NATURE OF THE RESIDUE, SORGHUM

Field Procedures

Plots 6 x 14 ft were prepared for sorghum at Ciba-Geigy research stations in MS (silty loam soil), IL (clay loam), and NY (loam). One half of each plot was planted with corn, and the other half with sorghum. Sorghum seed (a different variety at each plot) was planted in three rows at each plot. 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. In its Overview document (MRID 42547115), registrant notes that the maximum use rate on corn and sorghum has been reduced to 2.5 lb ai/A. The test substance was applied at each plot at sorghum height of 10 to 12 in. Control plots at each location were treated in the same manner, but with unlabeled atrazine. Sorghum leaves were sampled after application on the same day; sorghum forage samples were collected at 30 days and at silage stage (46-75 days), and sorghum grain and fodder were harvested at maturity (85-106 days). At maturity, grain was removed from sorghum, and stalks and leaves were collected as fodder samples. Control samples were harvested and processed in the same fashion as treated samples. Samples were collected by hand, placed into prelabeled plastic bags, and stored in a freezer. 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.

CBRS Comments, Sorghum Field Procedures

The previous review (CBRS No. 9167) concluded that corn and sorghum can be treated both pre- and post-emergent, and both the residue profile and magnitude will vary depending on the time of application. CBRS recommended a discussion of how the proposed studies will supplement available studies to provide data representative geographically, of soil types, and of registered uses. In response, registrant noted in its Overview document (MRID 42547115) that most of the previous metabolism studies have been conducted with pre-emergence application, and in these studies hydroxy metabolites represented the predominant residues. Registrant noted that post-emergence treatment was expected to result in a higher total radioactive residue, and in a more

complex mixture of metabolites, because of the potential for uptake of chloro triazines into treated leaves. In support of its comments, registrant referred to a previously submitted overview on atrazine metabolism, Report ABR-89060 (MRID 41209801). This previous report has already been reviewed (DEB No. 5783, 5/3/90, M.S. Metzger), and the review supports the claim that polar metabolites predominate with pre-emergence application.

With regard to the matter of geographic diversity with the field metabolism studies, registrant noted that each location represented a different soil type. In addition, the 10/90 DCI noted that for reregistration, magnitude of the residue studies would eventually be required in KS (26%) or NE (14%) and TX (22%), which collectively represent approximately 60% of 1985 U.S. sorghum grain production. For the purposes of geographic representation of field radiolabel studies only, the study in IL can be considered representative of the midwest, and the study in MS can be considered representative of the south. The study in NY was outside a major sorghum growing area of the U.S., and can be considered appropriate for diversity of field metabolism studies.

Conclusion 2a: For the purposes of these field radiolabel studies, studies with post-emergence application to sorghum in IL, MS, and NY are considered representative geographically, of soil types, and of registered uses.

In the previous review (CBRS No. 9167), CBRS recommended that a detailed protocol be submitted for review prior to initiation of these studies. The present submission (MRID 42547116) stated that the protocol was provided as Appendix A. However, Appendix A contains a single page listing deviations from the protocol. The protocol was not provided.

Conclusion 2b: The protocols for the sorghum metabolism studies should be provided.

While frozen storage conditions were described for storage at the performing laboratory, storage conditions between harvest and storage at the field site, and prior to shipment to the performing laboratory, are not immediately obvious. These data should be provided.

Conclusion 2c: Registrant should describe storage conditions for sorghum samples, including times and approximate temperatures of storage, between harvest and shipment to the performing laboratory.

Laboratory Analysis

Total radioactive residues (TRR) of sorghum samples were determined by combustion and liquid scintillation counting (LSC). Results are summarized in Table 5; forage, silage, and fodder samples are described above under Field Procedures. Sorghum leaf samples collected at day 0 after application were also analyzed; TRR in these samples was described as 193 to 567 ppm, but values at each location were not provided.

Table 5. TRR in Sorghum Samples.

Location	Sample	Days after Treatment	TRR, ppm
MS	Sorghum Forage	30	2.901
	Sorghum Silage	75	1.234
	Sorghum Fodder	98	0.907
	Sorghum Grain	98	0.392
IL	Sorghum Forage	30	0.875
	Sorghum Silage	46	0.277
	Sorghum Fodder	85	0.418
	Sorghum Grain	85	0.128
NY	Sorghum Forage	30	5.351
	Sorghum Silage	72	1.071
	Sorghum Fodder	106	1.043
	Sorghum Grain	106	0.033

Radioactive residues in sorghum samples were extracted by two similar methods, which were the same Methods I and II used for corn samples. Figure 2 above provides an outline for Method II. Tissues containing nonextractable residues and aqueous extract fractions were further treated by acid hydrolysis by refluxing with HCl at different concentrations for 4 h. The hydrolysis mixtures were filtered and rinsed with methanol. The filtrate and rinses were combined, neutralized with ammonium hydroxide, and partitioned with chloroform or ethyl acetate.

Extraction Method I was used for all sorghum samples from these studies; Method II was used for selected samples. The distributions of TRR using these methods are summarized in Table 6:

Table 6. Extraction of TRR from Sorghum Samples.

Sample		TRR, ppm	% TRR Extracted by Method I as:			
			Organic	Aqueous	Non-extracted	Total
MS	30 Day Forage	2.901	8.0	55.2	44.1	107.3
	Silage Forage	1.234	5.6	41.1	50.4	97.1
	Mature Fodder	0.907	4.3	47.0	47.3	98.6
	Mature Grain	0.392	1.0	31.6	68.0	100.6
IL	30 Day Forage	0.875	11.8	49.4	38.2	99.4
	Silage Forage	0.277	7.6	49.1	35.4	92.1
	Mature Fodder	0.418	6.5	46.1	51.3	103.8
	Mature Grain	0.128	2.6	23.6	72.7	98.9
NY	30 Day Forage	5.351	54.9	28.7	20.7	104.3
	Silage Forage	1.071	11.2	54.6	32.5	98.3
	Mature Fodder	1.043	10.0	48.5	45.0	103.5
	Mature Grain	0.033	2.4	16.2	85.2	103.8
Sample		TRR, ppm	% TRR Extracted by Method II as:			
			Organic	Aqueous	Non-extracted	Total
MS	30 Day Forage	2.901	13.8	56.6	20.8	91.2
	Mature Fodder	0.907	11.4	72.9	21.8	106.1
	Mature Grain	0.392	4.3	52.9	48.4	105.6
IL	30 Day Forage	0.875	14.8	42.3	21.1	78.2
	Mature Fodder	0.418	15.1	50.0	25.7	90.8
NY	30 Day Forage	5.351	45.0	42.0	12.1	99.1
	Mature Fodder	1.043	16.4	83.2	16.9	116.5

Table note: Not all samples were extracted by Method II.

Residue Identification

Fractions extracted by Method I were further analyzed to identify residues using techniques similar to those described for corn extracts. Organic fractions were analyzed by 2-dimensional thin layer chromatography (2-D TLC) using silica plates and two different combinations of solvent systems. Aqueous fractions

were analyzed by Aminex A-4 ion exchange chromatography. Peaks from TLC and Aminex chromatography were assigned based on similar mobilities with standards atrazine and its chloro and hydroxy metabolites indicated in Figure 1. Atrazine and chloro metabolites were identified in organic fractions. Hydroxy metabolites were chiefly identified in aqueous fractions, and to a lesser extent in organic fractions. Organic fractions in grain were not characterized, on the grounds that total radioactivity was low.

The major residue in most of the organic soluble fractions was Unknown 1, which showed a mobility on 2-D TLC similar to Unknown 1 in the corn samples. Unknowns 2, 3, and 4 were also detected with some samples. These unknowns were not further characterized, and registrant could not rule out the possibility that these were chloro triazines. Analysis of aqueous fractions by Aminex chromatography generally revealed at least 10 to 13 components. Peaks were identified corresponding to standards for the atrazine hydroxy metabolites indicated in Figure 1, and identification of these peaks as the hydroxy metabolites was confirmed by HPLC analysis. Metabolite GS-17794 represented a dominant metabolite in several of the sorghum samples. The assignment of residues from Extraction Method I is summarized in Table 7. The assignment of residues in organic fractions only is summarized in Table 7A, which indicates that known hydroxy metabolites did not represent a major portion of the organic soluble residue for any sorghum sample.

Major unknowns identified by Aminex chromatography were Peak No. 1, which represented up to 20% of TRR in forage and fodder, and Peak No. 7, which represented up to 11% of TRR in forage. Other unknown peaks on Aminex chromatography represented no more than 5.4% of TRR. The height of Peak No. 7 was seen to decrease with time after treatment, and this was accompanied by an increase in Peak No. 1 with time. DEAE Sephadex A-25 anion exchange chromatography indicated that Peak No. 1 consisted of at least four components. The major Sephadex peak represented 9.3% of TRR; when fractions from this peak were hydrolyzed in 6 N HCl for 20 h, analysis by Aminex chromatograpy indicated three peaks, one with mobility of G-34048 and representing about a third of recovered radioactivity. Purification of this peak and analysis by mass spectrometry revealed a profile in good agreement with that of a G-34048 standard.

Peak No. 7 from Aminex chromatography was shown to have similar mobility as the lanthionine conjugate of atrazine (see Figure 3 for structure). Peak No. 7 was purified and examined by fast atom bombardment mass spectrometry. The resulting negative ion spectrum contained strong peaks consistent with those of a lanthione-atrazine conjugate standard. However, the spectrum contained other strong peaks not seen with the standard, suggesting that Peak No. 7 includes additional components. When

Peak No. 7 was hydrolyzed in 6 N HCl for 20 h, Aminex chromatography indicated three peaks, including one with the mobility of G-34048, similar to those seen after hydrolysis of Peak No. 1. Strong acid hydrolysis of the lanthionine-atrazine conjugate standard and analysis by Aminex chromatograhy also produced the same three peaks.

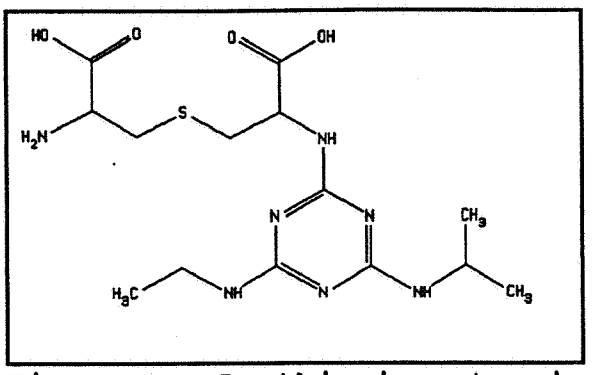

Figure 3. Lanthionine-atrazine conjugate

Registrant did not attempt to assign % TRR values to the lanthionine-atrazine conjugate. However, the work presented is sufficient to identify this conjugate as a component of Aminex Peak No. 7. Registrant postulated that with time, the conjugate was converted to the components of Peak No. 1, which may represent other 2-position derivatives of the 4-ethylamino-6-isopropyl amine-2-triazine moiety. Registrant postulated that the lanthionine conjugate is formed through conjugation of atrazine with glutathione, followed by additional metabolic steps. However, only the lanthionine conjugate was identified in this submission.

Fractions obtained from Extraction Method II were also analyzed further. Although Method II in most cases increased the % TRR extracted in organic fractions, these fractions were not further characterized. Aqueous fractions were analyzed by Aminex chromatography and hydroxy metabolites were assigned as described for Extraction Method I. Method II resulted in increased assignment of aqueous soluble residues as the hydroxy metabolites, presumably by the release of some conjugated residues. A comparison between Methods I and II of residues assigned as hydroxy metabolites is summarized in Table 8. With the exception of peaks assigned to GS-17794, the peaks from Aminex chromatography were not analyzed by a second method to confirm the residue assignment. When putative peaks of GS-17794 were analyzed by one-dimensional TLC, other components were revealed; in no case did GS-17794 represent more than 50% of the residue obtained from its putative Aminex peak. Thus, Extraction Method II may release compounds other than the hydroxy metabolites, which show the same mobility during Aminex chromatography. The performing laboratory advised that caution should be used when interpreting analytical results using Extraction Method II.

The nonextracted residue from MS grain (68% TRR) after treatment by Method I was further treated by reflux in 0.1 N HCl, the extract was partitioned with chloroform, and the aqueous soluble phase was analyzed. This hydrolysis extracted additional known

Table 7. Assignment of Residues in Sorghum, Extracted by Method I.
(Table Notes are the same as for Table 3)

Residue	Percent of TRR assigned by location and sample:											
	MS				IL				NY			
	30-Day Forage	Silage Forage	Mature Fodder	Mature Grain	30-Day Forage	Silage Forage	Mature Fodder	Mature Grain	30-Day Forage	Silage Forage	Mature Fodder	Mature Grain
Organic Fraction	(8.0)	(5.6)	(4.3)	(1.0) NA	(11.8)	(7.6)	(6.5)	(2.6) NA	(54.7)	(11.2)	(10.0)	(2.4) NA
Atrazine	0.7	0.2	0.1		2.3	0.1	0.3		49.6	4.7	5.8	
G-28273	0.2	0.2	<0.1		0.1	0.3	0.1		<0.1	0.2	0.1	
G-28279	0.6	0.2	0.2		0.4	0.7	0.2		0.3	0.3	0.3	
G-30033	0.9	0.2	0.1		1.2	0.4	0.5		0.7	1.4	0.7	
Unknowns 1-4	2.2	3.5	1.7		2.9	1.1	3.6		0.9	2.3	1.6	
Hydroxy:												
GS-17791	1.0	0.8	0.9	1.2	1.1	0.5	0.6	0.8	0.4	0.5	0.9	0.4
GS-17792	0.7	1.5	2.1	1.9	0.4	1.3	1.3	1.1	0.3	0.3	1.3	1.8
GS-17794	7.8	4.9	5.7	3.5	4.4	3.1	4.2	0.7	2.4	4.3	4.2	0.4
G-34048	0.8	0.1	0.4	<0.1	0.8	1.5	0.1	0.3	0.6	0.2	0.4	0.3
Peak No. 7	11.2	1.6	1.8	0.8	11.3	6.5	5.5	0.9	9.9	9.2	5.1	0.3
Total known hydroxy:	10.3	7.3	9.1	6.6	6.7	6.4	6.2	2.9	3.7	5.3	6.8	2.9
Total, known compounds:	12.7	8.1	9.5	6.6	10.7	7.9	7.3	2.9	54.3	11.9	13.7	2.9

Table 7A. Assignment of Residues in Sorghum, Organic Fractions from Method I.

Residue	Percent of TRR assigned by location and sample:								
	MS			IL			NY		
	30-Day Forage	Silage Forage	Mature Fodder	30-Day Forage	Silage Forage	Mature Fodder	30-Day Forage	Silage Forage	Mature Fodder
Total Organic Fraction	(8.0)	(5.6)	(4.3)	(11.8)	(7.6)	(6.5)	(54.7)	(11.2)	(10.0)
Atrazine	0.7	0.2	0.1	2.3	0.1	0.3	49.6	4.7	5.8
G-28273	0.2	0.2	<0.1	0.1	0.3	0.1	<0.1	0.2	0.1
G-28279	0.6	0.2	0.2	0.4	0.7	0.2	0.3	0.3	0.3
G-30033	0.9	0.2	0.1	1.2	0.4	0.5	0.7	1.4	0.7
Unknowns 1-4	2.2	3.5	1.7	2.9	1.1	3.6	0.9	2.3	1.6
Hydroxy:									
GS-17792	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1
GS-17794	0.1	0.1	0.1	<0.1	0.1	<0.1	0.1	<0.1	<0.1
G-34048	0.3	0.1	0.1	0.5	1.5	0.1	0.4	0.2	<0.1
Total known hydroxy:	0.4	0.2	0.1	0.5	1.6	0.1	0.6	0.2	<0.1
Total, known compounds:	2.8	1.0	0.5	4.5	3.1	1.2	51.2	6.8	6.9

Table notes: See Figure 1 for structures. Organic fractions from grains were not analyzed. GS-17791 was not detected in any organic fractions.

Table 8. Comparison of Residue Assignment by Extraction Method I or Method II.

Residue	% of TRR in Sorghum Samples Assigned to:													
	MS						IL				NY			
	30-Day Forage		Mature Fodder		Mature Grain		30-Day Forage		Mature Fodder		30-Day Forage		Mature Fodder	
	Method I	II	I	II	I	II	I	II	I	II	I	II	I	II
Organic Fraction	8.0	13.8	4.3	11.4	1.0	4.3	11.8	14.8	6.5	15.1	54.9	45.0	10.0	16.4
Aqueous Fraction	55.2	56.6	47.0	72.9	31.6	52.9	49.4	42.3	46.1	50.0	28.7	42.0	48.5	83.2
Within Aqueous Fraction:														
GS-17791	1.0	3.6	0.9	5.1	1.2	7.1	1.1	1.2	0.6	4.5	0.4	0.7	0.9	4.7
GS-17792	0.7	1.4	2.1	4.0	1.9	2.1	0.4	0.7	1.3	2.5	0.2	ND	1.3	2.7
GS-17794	7.7	13.9	5.7	11.4	3.5	4.8	4.4	12.8	4.2	7.9	2.3	9.0	4.2	13.7
G-34048	0.5	2.0	0.3	ND	ND	3.4	0.3	1.3	ND	0.8	0.2	19.1	0.4	6.8
Total, Hydroxy in Aqueous	9.9	20.9	9.0	20.5	6.6	17.4	6.2	16.0	6.1	15.7	3.1	28.8	6.8	27.9

Table notes: ND = non-detectable

hydroxy metabolites representing 20.3% of TRR. When the nonextracted residue from this initial acid hydrolysis was treated by reflux in 1.0 N HCl, an additional 5.1% of TRR was released as known hydroxy metabolites. Nonextracted residues from other samples after treatment by Method I or Method II were also further treated by acid hydrolysis; recovery of the known hydroxy metabolites was less dramatic than for the MS grain. Further analysis of the organic phases from these acid hydrolysis treatments was not reported.

Aqueous fractions of sorghum fodder samples from IL and NY were incubated with ß-glucosidase. Little difference was observed in the profile from an Aminex A-4 column in extracts before or after treatment, indicating that glucose conjugates did not represent a significant portion of the total residue. Sorghum grain samples from MS were extracted by Method I in November 1991, 2 mo after mature harvest, and in March 1992, 7 mo before the termination of laboratory analysis. Aminex A-4 chromatography profiles of the aqueous extracts were generally consistent, indicating adequate storage stability over the course of the study.

CBRS Comments, Residue Identification in Sorghum

This submission identified in sorghum parent atrazine, the chloro and hydroxy metabolites indicated in Figure 1, and the lanthionine conjugate indicated in Figure 3; other residues were not identified. Therefore, no data were provided to indicate that the total radioactive residue may include metabolites other than those consisting of the triazine ring with a substituent. In the absence of additional data on the nature of the residue, the earlier conclusion that exposure assessment should be conducted on the basis of the total radioactive residue is not altered.

Conclusion 2d: No data for sorghum were provided which alter the position of the HED Metabolism Committee that exposure assessment for atrazine should be conducted on the basis of the total radioactive residue (Memo, 8/7/92, M.S. Metzger).

Anticipated residues for atrazine were previously determined from data obtained from metabolism studies in the greenhouse and in the field, and were 0.13 ppm in sorghum grain and 2.02 ppm in sorghum forage (DEB No. 5783, 5/3/90, M.S. Metzger). In the present submission, average residues across studies in MS, IL, and NY were 3.04 ppm in 30-day sorghum forage, 0.86 ppm in sorghum silage forage, 0.79 ppm in sorghum fodder, and 0.18 ppm in sorghum grain (see Table 5).

Conclusion 2e: Averaging residues from the present studies with those from previous studies would not be expected to produce a significant change in anticipated residues for sorghum

commodities. Revised anticipated residues for corn and sorghum will be provided in a separate memorandum.

Previous review noted that the composition of the residue in plants can vary with crop and crop commodity (CBRS No. 9167, 1/22/92, M.S. Metzger). Such variability was also encountered in the present submission, and field location and extraction method were indicated as additional factors influencing variability. In the present studies, the percent of TRR represented by organic soluble residues ranged from 1.0% to 4.3% in sorghum grain and from 4.3% to 54.9% in sorghum forage and fodder; the percent of TRR assigned to known hydroxy metabolites ranged from 2.9% to 17.4% in sorghum grain and from 5.3% to 28.8% in sorghum forage and fodder (see Tables 7 and 8). Previous review concluded that in cases where the entire residue containing the triazine ring was not measured, conservative assumptions will have to be used in conducting exposure assessment.

Conclusion 2f: The present submission indicates variability in the composition of the total residue in sorghum with location and commodity, and variability in residues detected with the method of extraction. In the present studies, the percent of TRR represented by organic soluble residues ranged from 1.0% to 4.3% in sorghum grain and from 4.3% to 54.9% in sorghum forage and fodder; the percent of TRR assigned to known hydroxy metabolites ranged from 2.9% to 17.4% in sorghum grain and from 5.3% to 28.8% in sorghum forage and fodder. If a marker method is ultimately accepted, conservative assumptions will be necessary for determining appropriate tolerance levels.

Residues of parent atrazine and the chloro metabolites are known to be extractable into organic solvents. The present submission for sorghum identified Unknowns 1 through 4 as significant components of the organic soluble fractions, and registrant could not rule out the possibility that these unknowns were chloro triazines. In some cases, the Unknowns combined represented a greater % of TRR than did combined parent and known chloro metabolites (see Table 7). In addition, the present submission found with a corn sample that acid hydrolysis of the unextracted residue released a chloro triazine metabolite (see above, CBRS Comments, Residue Identification in Corn). Although unextracted residues in sorghum were treated with acid hydrolysis, analysis of the subsequent organic soluble fractions was not reported.

Conclusion 2g: The present submission identified in sorghum oganic soluble unknowns which may be chloro triazines, and the ability of mild acid hydrolysis to release chloro triazines from unextracted residues was not reported. In the absence of additional data on the identity of metabolites, organic soluble residues extracted under mildly acidic conditions should be assumed to represent chloro triazine compounds.

With regard to the hydroxy metabolites, the performing laboratory noted that treatment by Extraction Method II, under mildly acidic conditions, could release compounds different from the known hydroxy metabolites, but which showed the same mobility during Aminex chromatography as the hydroxy metabolites. The performing laboratory noted that caution should be used when interpreting the analytical results using Extraction Method II. Because the identity of the hydroxy metabolites obtained from Extraction Method I were confirmed by a second analytical method, the assignment of hydroxy metabolites under this method should be assumed to be more reliable.

Conclusion 2h: The present submission found that if sorghum samples were extracted under mildly acidic conditions, aqueous soluble fractions contained residues different from the known hydroxy metabolites, but which showed similar chromatographic mobility as these hydroxy metabolites. In the absence of additional data on the identity of metabolites extracted under mildly acidic conditions, extraction by methanol:water (Extraction Method I) should be assumed to provide a more reliable assignment of the known hydroxy metabolites.

Recommendations, Nature of the Residue: Before the data submitted from field metabolism studies of corn and sorghum can be accepted, the registrant must resolve the deficiencies identified in Conclusions 1c, 1d, 2b, and 2c. As indicated in Conclusions 1e and 2d, exposure assessment in corn and sorghum should continue to be conducted on the basis of the total radioactive residue.

ANALYTICAL METHOD

Reregistration Requirements

The Residue Chemistry Chapter (7/25/83) concluded that adequate analytical methods were available for data collection pertaining to parent atrazine residues in plants and animals. Although adequate methods were available for enforcement of most plant tolerances, data gaps still existed for the enforcement of tolerances for animal commodities, and for the enforcement of tolerances of the chloro metabolites G-28273, G-28279, and G-30033. The Second Round Review Residue Chemistry Chapter (10/18/88) concluded that the nature of the residue in plants and animals was not adequately described, and methods to be used in the future for data collection and tolerance enforcement would be determined following receipt of the requested metabolism data.

A subsequent review (DEB No. 5873, 5/3/90, M.S. Metzger) noted that all metabolites containing the intact triazine ring were now considered of toxicological concern. The Agency subsequently issued a DCI, received by registrant in 10/90, which superceded the residue chemistry requirements of all previous DCIs and any

other agreements entered into with the Agency pertaining to such requirements. This DCI required that residue analytical methods must be developed and validated which will quantify all atrazine metabolites containing an intact triazine ring in or on plant commodities and in animal commodities. The 10/90 DCI also required that successful confirmatory trials must be conducted by an independent laboratory for i) GLC method AG-484 using field corn grain, sweet corn K+CWHR, and corn forage; ii) GLC method from EN-CAS Report 86-284 [sic] using eggs and poultry tissues; iii) GLC method AG-476 using meat and meat byproducts; and iv) GLC methods AG-436 [sic] and AG-496 for milk. The 10/90 DCI also required that representative plant and animal tissue samples bearing residues of all atrazine metabolites with an intact triazine ring must be subjected to analysis by multiresidue protocols I and III from PAM Vol. I, Appendix II.

The HED Metabolism Committee addressed issues related to triazine chemicals at a meeting of 7/9/92 (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. 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, 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.

Methods for Marker Residues

To support the development of analytical methodology capable of measuring metabolites that can be used as markers, and to validate those methods using radiolabeled samples from metabolism studies, registrant Ciba-Geigy provided the following documents with the present submission:

Atrazine: Analytical Method for the Determination of G-34048 and GS-17794 in Crops by High Performance Liquid Chromatography with Column Switching, Analytical Method AG-596, Ciba-Geigy Corporation, June 26, 1992 (MRID 42547119).

Validation of Analytical Method AG-596 by the Analysis of ^{14}C-Atrazine Treated Corn and Sorghum Raw Agricultural Commodities: 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-92025, October 27, 1992 (MRID 42547118).

Document MRID 42547119 describes Method AG-596. With this method, a crop sample is refluxed with methanol:water (1:3) for 1 h. The extract and solid pellet are acidified with HCl, then 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 is taken and the methanol removed by evaporation. The remaining liquid is diluted with water and loaded onto a Sephadex G-10 gel chromatography column. The volume loaded and rinse in methanol are collected. The eluate is acidified with HCl and loaded onto an AG50W-8X cation exchange column. The column is rinsed with methanol and dilute ammonium hydroxide in methanol, then eluted with ammonium hydroxide in aqueous methanol. This eluate is evaporated to dryness, and the sample is reconstituted in aqueous 0.3% ammonium acetate for analysis by HPLC and UV detection.

HPLC analysis of GS-17794 and G-34048 is conducted on a C8 column switched to an SCX column, with a different mobile phase used for each compound. For either compound, column one is Spherisorb C8, and column 2 is Supelcosil LC-SCX. For detection of GS-17794, mobile phase 1 is buffer:methanol (90:10), and mobile phase 2 is buffer:methanol (80:20); for detection of G-34048, mobile phase 1 is buffer:methanol (60:40), and mobile phase 2 is buffer:methanol (50:50). Buffer for all mobile phases is 0.3% aqueous ammonium acetate, acidified to pH 5.0 with acetic acid. The detected compounds migrate as individual peaks; residues are quantitated based on peak heights compared with those of standards.

Registrant provided representative chromatograms from analysis of fortified samples. It should be noted that for several samples, potentially interfering peaks from control matrices migrate with similar mobilities as the standard. As Appendices to the document MRID 42547119, registrant included reports on Methods AG-571 and AG-572, from which Method AG-596 was developed.

Document MRID 42547118 was submitted to validate Methods AG-484 and AG-596, using radiolabeled samples from corn and sorghum field metabolism studies. 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). Method AG-596 is described above. 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 two 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 a DB-WAX column. The limits of detection for each compound are 0.001 ppm in corn grain, and 0.01 ppm in sorghum grain. Registrant validated each method using control samples of corn and sorghum from each location for the metabolism studies, fortified with each of the residues to be detected. Table 9 summarizes recovery data for Method AG-484, and Table 10 summarizes recovery data for Method AG-596, at the lowest fortification levels examined with each method:

Table 9. Recoveries from Fortified Samples with Method AG-484.

Sample, Fortification, ppm	Site	% Recovery for metabolite:			
		Parent	G-30033	G-28279	G-28273
Corn Forage, 0.05	MS	106	102	89	88
	IL	89	88	102	95
	NY	120	123	91	60
Corn Silage, 0.05	MS	105	103	92	114
	IL	101	117	114	64
	NY	110	116	94	106
Corn Fodder, 0.05	MS	80	93	95	66
	IL	94	94	93	89
	NY	117	110	116	114
Corn Grain, 0.001	MS	140	64	77	65
	IL	92	111	69	118
	NY	95	81	59	76
Sorghum Forage, 0.05	MS	110	104	125	103
	IL	88	107	82	88
	NY	102	107	90	92
Sorghum Silage, 0.05	MS	125	118	106	106
	IL	101	94	77	70
	NY	114	129	116	98
Sorghum Fodder, 0.05	MS	98	90	92	80
	IL	86	92	88	126
	NY	121	111	108	92
Sorghum Grain, 0.01	MS	114	90	109	77
	IL	135, 105	83, 90	72, 89	87
	NY	99	123	123	90

Table 10. Recoveries from Fortified Samples with Method AG-596.

Sample	Site	% Recovery for metabolite: G-34048	GS-17794
Corn Forage	MS	69	86
	IL	126	128
	NY	83	100
Corn Silage	MS	87	94
	IL	113	127
	NY	89	96
Corn Fodder	MS	77	75
	IL	84	94
	NY	103	100
Corn Grain	MS	82	85
	IL	66	85
	NY	76, 64	88, 85
Sorghum Forage	MS	81	109
	IL	95	116
	NY	72	95
Sorghum Silage	MS	73	114
	IL	88	88
	NY	84	81
Sorghum Fodder	MS	78	103
	IL	74	88
	NY	98	92
Sorghum Grain	MS	83	74
	IL	84	118
	NY	76	79

Table notes:
Fortification was at 0.02 ppm for all samples indicated.

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 11 summarizes the residues detected from these samples. Table 11 includes a column designated Maximum Expected residues for comparison with the residues accounted for by the marker metabolites detected; this column was obtained from the data in Tables 3 and 7. For samples other than grains, the maximum residues expected as % TRR were calculated from the combined magnitudes of the marker compounds as reported in Tables 3 and 7. For grain samples, the maximum residues expected were calculated from the total residue in the organic fraction, plus the combined magnitudes of the marker compounds G-34048 and GS-17794. In Table 11, for samples with combined residues nondetectable by Methods AG-484 and AG-596, Maximum Expected values are expressed as both % TRR and ppm.

Table 11. Recoveries from Field Metabolism Samples by Methods AG-484 and AG-596.

Site	Sample	TRR, ppm	Detected by AG-484, ppm	Detected by AG-596, ppm	Total % TRR Detected	Maximum Expected, % TRR (ppm)
MS	Corn Forage	0.694	≤0.20	0.36	52	27.7
	Corn Silage	0.660	≤0.20	0.173	26	23.1
	Corn Fodder	0.850	≤0.20	0.178	21	15.3
	Corn Grain	0.045	≤0.004	≤0.048	ND	3.2 (0.001)
IL	Corn Forage	0.466	≤0.20	0.104	23	25.6
	Corn Silage	0.710	≤0.20	0.143	20	29.8
	Corn Fodder	1.809	≤0.20	0.48	26	20.3
	Corn Grain	0.071	≤0.004	≤0.048	ND	7.6 (0.005)
NY	Corn Forage	2.84	1.14	0.37	53	55.7
	Corn Silage	0.499	≤0.20	0.063	9	22.2
	Corn Fodder	1.549	≤0.20	0.158	10	17.9
	Corn Grain	0.034	≤0.004	≤0.048	ND	12.0 (0.004)
MS	Sorghum Forage	2.901	≤0.202	0.22	9	11.0
	Sorghum Silage	1.234	≤0.207	0.079	11	5.8
	Sorghum Fodder	0.907	≤0.20	0.054	6	6.5
	Sorghum Grain	0.392	≤0.04	≤0.048	ND	4.5 (0.018)
IL	Sorghum Forage	0.875	≤0.20	≤0.048	ND	9.2 (0.080)
	Sorghum Silage	0.277	≤0.20	≤0.048	ND	6.1 (0.017)
	Sorghum Fodder	0.418	≤0.20	≤0.048	ND	5.4 (0.023)
	Sorghum Grain	0.128	≤0.04	≤0.048	ND	3.6 (0.005)
NY	Sorghum Forage	5.351	2.4	0.135	47	53.6
	Sorghum Silage	1.071	≤0.20	0.041	8	11.1
	Sorghum Fodder	1.043	≤0.20	≤0.048	ND	11.5 (0.120)
	Sorghum Grain	0.033	≤0.04	≤0.048	ND	3.1 (0.001)

Table notes: Results for Methods AG-484 and AG-596 were the average of three determinations; residues detected by AG-596 are expressed as atrazine equivalents.
Maximum Expected values were calculated from Tables 3 and 7; see text for details. For samples with combined residues nondetectable by Methods AG-484 and AG-596, Maximum Expected values are expressed as both % TRR and ppm.
ND = ≤ limits of detection.

The data in MRID 42547118 allow an assessment of the radiovalidation of each method. With Method AG-484, there were only a few positive detections of chloro triazines using samples from the field metabolism studies; there were no positive detections at all of metabolites G-30033 and G-28279. Table 12 indicates the results of positive detections of atrazine and G-28273. The residues detected are compared with residues expected, based on the data in Tables 1 and 3 for corn, or Tables 5 and 7 for sorghum. Table 13 provides radiovalidation data for Method AG-596. As with Table 12, only positive detections for one or more metabolite are indicated. In Table 13, residues expected were calculated in the same manner as described for Table 12.

Table 12. Method AG-484, Positive Detections in Field Metabolism Samples.

	Atrazine, ppm:		G-28273, ppm:	
Sample	Detected	Expected	Detected	Expected
NY Corn Forage	1.14	1.23	≤0.05	0.003
MS Sorghum Forage	≤0.05	0.020	0.052	0.006
MS Sorghum Silage	0.057	0.002	≤0.05	0.002
NY Sorghum Forage	2.40	2.65	≤0.05	≤0.005

Table notes:
Only positive detections are included; results are the average of three determinations.
Expected residues were determined from Tables 1 and 3, or 5 and 7.

Table 13. Method AG-596, Positive Detections in Field Metabolism Samples.

Site	Sample	G-34048:			GS-17794:		
		Detected, ppm	Expected, ppm	Detected÷ Expected	Detected, ppm	Expected, ppm	Detected÷ Expected
MS	Corn Forage	0.107	0.056	1.91	0.188	0.128	1.47
	Corn Silage	≤0.02	0.019	ND	0.135	0.131	1.03
	Corn Fodder	0.021	0.005	4.20	0.138	0.124	1.11
IL	Corn Forage	0.033	0.028	1.18	0.053	0.078	0.68
	Corn Silage	0.045	0.058	0.78	0.073	0.141	0.52
	Corn Fodder	0.096	0.054	1.78	0.290	0.306	0.95
NY	Corn Forage	0.186	0.105	1.77	0.127	0.230	0.55
	Corn Silage	≤0.02	0.010	ND	0.049	0.093	0.53
	Corn Fodder	0.021	0.031	0.68	0.111	0.223	0.50
MS	Sorghum Forage	0.045	0.023	1.96	0.129	0.226	0.57
	Sorghum Silage	≤0.02	0.001	ND	0.061	0.060	1.02
	Sorghum Fodder	≤0.02	0.004	ND	0.037	0.052	0.71
NY	Sorghum Forage	0.095	0.032	2.97	0.024	0.128	0.19
	Sorghum Silage	≤0.02	0.002	ND	0.021	0.046	0.46

Table notes:
Only positive detections of one or both metabolites are included; results are the average of three determinations.
Expected residues were determined from Tables 1 and 3 for corn, or 5 and 7 for sorghum.
ND = not detectable with Method AG-596

CBRS Comments, Methods for Marker Residues

Considering that they were obtained at the limits of determination, the recoveries from fortified samples in Tables 9 and 10 represent acceptable ranges.

Conclusion 3a: 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, 0.001 ppm in corn grain, 0.01 ppm in sorghum grain, and 0.05 ppm in other corn and sorghum commodities, for each of parent atrazine, G-30033, G-28279, and G-28273. The limits of determination for Method AG-596 were the lowest levels analyzed, 0.02 ppm in corn and sorghum commodities, for G-34048 and GS-17794.

In the previous review of Ciba-Geigy's response to the DCI of October 1990, CBRS commented on registrant's proposal to develop a marker method (CBRS No. 9167, 1/22/92, M.S. Metzger). This review noted two problems in developing a marker method, variability in the composition of the total residue, and the need to detect marker metabolites with very low limits of detection. With regard to the first problem, the previous review noted that the composition of the total residue from atrazine use can vary with crop, with commodity, and PHI. Data from the present submission indicate that location represents an additional source of variability, and residue detected can vary with the method of extraction (see Conclusions 1g and 2f, and related discussion above).

Registrant's proposal is to use as marker compounds parent atrazine, G-30033, G-28279, and G-28273, which are detected by Method AG-484; and G-34048 and GS-17794, which are detected by Method AG-596. In the field metabolism studies provided with the present submission, these marker compounds represented over 50% of the total radioactive residue, most of which was atrazine, in NY 30-day forage samples for corn and sorghum. In other samples, the six markers represent up to 30% TRR in corn silage forage, up to 20% TRR in corn fodder, and no more than 12% TRR in corn grain (see Table 3 and the Maximum Expected column in Table 11). With sorghum, field metabolism studies indicate that the marker compounds represent no more than 11% TRR in silage forage and fodder, and less than 5% TRR in grain (see Table 7 and the Maximum Expected column in Table 11).

The second difficulty in developing an analytical method using marker compounds is the need for low limits of detection. Previous review noted that the current risk assessment for atrazine from corn grain shows a cancer risk of 8.4×10^{-6}, assuming anticipated residues of 0.1 ppm (CBRS No. 9167, 1/22/92, M.S. Metzger). If a marker method detected a metabolite representing approximately 10% of TRR, then a limit of detection of 1 part per billion (ppb) would be necessary to demonstrate a risk from corn grain of less than 10^{-6}. Since the six marker compounds detected by the proposed methods represent no more than 12% TRR in corn grain, a combined limit of detection of no more than 1 ppb would be required. However, the combined limit of detection for the six markers is on the order of 0.05 ppm in corn grain.

Recommendation: CBRS acknowledges that the present submission represents considerable scientific effort, and the work appears to be competently done. However, CBRS must defer a conclusion on the marker method. The HED Metabolism Committee has concluded that if data indicating that hydroxyatrazine is not carcinogenic were reviewed and accepted, then exposure assessment and tolerance expression for atrazine would include only parent and chloro metabolites. If such an alteration in the residues of

concern were to occur, then it would seem feasible that methods presently available could be developed for tolerance enforcement. CBRS therefore reserves judgement on the proposed marker methods, pending review of data on the carcinogenicity of hydroxyatrazine.

The data provided in the present submission do allow some conclusions to be drawn on radiovalidation. Table 12 indicates that for Method AG-484, the only samples where positive detections were obtained were those with significant atrazine residues, or those where interferring effects caused false positive detections as trace residues. Other samples served to confirm the limits of detection of this method. The samples in Table 12 are sufficient to validate the ability of the method to detect residues of parent atrazine in the range of 1-2 ppm.

Conclusion 3b: Radiovalidation data for Method AG-484 are sufficient to validate the ability of the method to detect residues of parent atrazine in the range of 1-2 ppm.

Radiovalidation data were also provided for Method AG-596. The data in Table 13 indicate that with two exceptions, the method was able to detect metabolite GS-17794 in field metabolism samples in the range of 50-150% of residues determined by field metabolism studies in corn and sorghum. Validation of the method's ability to detect metabolite G-34048 was less satisfactory. As Table 13 indicates, for the majority of samples, the method detected residues greater (in some cases considerably greater) than those determined by the metabolism study. Method AG-596 uses a mild acid hydrolysis extraction, and the data above indicate that such conditions can release residues with similar chromatographic mobilities as the known hydroxy metabolites, but which in fact are different compounds (see for example, Conclusion 2h and discussion under Nature of the Residue, Sorghum). It appears that such additional compounds are not significant in interfering with identification of GS-17794, which is the major single metabolite in many of the corn and sorghum samples (see Tables 3 and 7). However, the release of such interfering compounds may have the effect of inflating the residues of G-34048 detected by Method AG-596. Because there has not been a precise determination of the known hydroxy metabolites released from metabolism samples under mild acid hydrolysis, Method AG-596 is not considered radio-validated for compound G-34048.

Conclusion 3c: Radiovalidation data for Method AG-596 are sufficient to validate the method's ability to measure residues of compound GS-17794 at levels greater than 0.02 ppm in corn and sorghum.

Conclusion 3d: Method AG-596 uses mild acid hydrolysis, which releases compounds that could artificially inflate detected residues of the hydroxy metabolites (see, for example, Conclusion

2h). Because there has not been a precise determination of the known hydroxy metabolites released from metabolism samples by mild acid hydrolysis, Method AG-596 is not considered radio-validated for compound G-34048.

Independent Laboratory Validation

In its cover letter to the present submission, registrant Ciba-Geigy reported that Methods AG-463, AG-496, and EN-CAS 86-264 failed the "ruggedness" trials in 1989 and were rewritten and validated with modifications by Ciba-Geigy. The new version of the methodology in milk is AG-496A; the new version of EN-CAS 86-264 is Method AG-593. Both methods have undergone validation by a second laboratory, and these validations are provided with this submission.

Registrant noted that a recent communication (FAX of 9/18/92) from the Agency indicated that the requirement for hydroxytriazine methodology in animal commodities is reserved until the toxicological concern of the hydroxytriazine residues is better established. CBRS acknowledges that this communication granted that the requirement for methodology to measure hydroxytriazine residues in animal commodities will be reserved pending review of the hydroxyatrazine chronic feeding/oncogenicity study. If the study indicates that the hydroxy metabolite is carcinogenic, then the requirement for hydroxytriazine methodology for animal commodities may be initiated.

Conclusion 4a: CBRS acknowledges that the requirement for methodology to measure hydroxytriazine residues in animal commodities (Guideline 171-4(d)) is reserved, pending review of the hydroxyatrazine chronic feeding/oncogenicity study.

In addition, the registrant reported documents that have previously been submitted: Methods AG-484 and AG-476 have passed "ruggedness" trials, and these trials were submitted in 1990 as MRIDs 41397102 and 41397103, respectively. A multiresidue study for atrazine, simazine, and their individual and common metabolites (MRID 41423401) was submitted in March of 1990 as part of the response to the 1988 DCI. It should be noted that CBRS has already examined these documents, and concluded that they only contained information relevant to the combined residues of atrazine and its chlorometabolites; review was declined because the documents did not contain data on the total residue of concern (DEB Nos. 6796, 6797, 7/26/90, M.S. Metzger). At present, the total residue of concern consists of parent and all metabolites containing a triazine ring with a substituent. The components of this total residue of concern still are not adequately defined.

Conclusion 4b: CBRS has previously declined review of data on independent laboratory validation of Methods AG-484 (MRID 41397102) and AG-476 (MRID 41397103) and on multiresidue protocols (MRID 41423401), because data were not provided for the total residue of concern.

In response to requirements of the 10/90 DCI regarding independent laboratory validation of analytical methods, registrant Ciba-Geigy has provided the following documents with the present submission:

Atrazine: Determination of Atrazine, G-28279, G-30033, and G-28273 Residues in Fresh and Sour Milk Using a Strong Cation Exchange Column Isolation and Cleanup, Method AG-496A, Ciba-Geigy Corporation, May 25, 1990 (MRID 42547120).

Atrazine: Independent Laboratory Confirmation of the Tolerance Enforcement Method by EPA PR Notice 88-5 and for the Determination of Atrazine, G-28279, G-30033, and G-28273 Residues in Fresh and Sour Milk Using a Strong Cation Exchange Column and Cleanup. Analytical Method AG-496A, Cyal, Inc., November 9, 1990 (MRID 42547121).

Atrazine: Analytical Method for the Determination of Residues of Atrazine, G-28273, G-28279, and G-30033 in Poultry Tissues and Eggs by Gas Chromatography Including Validation Data, Method AG-593, Ciba-Geigy Corporation, May 4, 1992 (MRID 42547122).

Atrazine: Independent Laboratory Confirmation of the Tolerance Enforcement Method by EPA PR Notice 88-5 for the Determination of Residues of Atrazine, G-28273, G-28279, and G-30033 in Poultry Tissues and Eggs by Gas Chromatography Analytical Method AG-593, Cyal, Inc., November 16, 1992 (MRID 42547123).

The document MRID 42547120 contains a description of Method AG-496A, which registrant indicates supercedes methods AG-496 and AG-463. With this method, protein is precipitated from milk samples by addition of acetone, vortexing, and centrifugation. The supernatant is decanted into a flask. Acetone is removed by evaporation and the aqueous solution applied to a strong cation exchange column (Mega Bond Elut SCX, Analytichem). The column is washed with water and eluted with 50% 0.5 M aqueous NaCl and 50% methanol. Methanol is removed from the eluate by evaporation and the remaining solution extracted three times with ethyl acetate; the ethyl acetate is concentrated to dryness. The resulting residue is dissolved in tolune:methanol (96:4) and analyzed by capillary (DB-5 column, J&W) gas chromatography with nitrogen-phosphorus detection. The detected compounds migrate as individual GC peaks, and residues are quantitated based on peak heights compared with standards.

Registrant Ciba-Geigy provided recovery data for milk samples fortified at 0.01 and 0.05 ppm of each compound. Table 14 summarizes recovery data for the two samples for each compound fortified at 0.01 ppm:

Table 14. Recoveries from Fortified Samples with Method AG-496A.

Compound	Sample	Recoveries, %
Atrazine	Fresh Milk	91, 98
	Sour Milk	96, 91
G-30033	Fresh Milk	92, 102
	Sour Milk	105, 94
G-28279	Fresh Milk	109, 133
	Sour Milk	134, 114
G-28273	Fresh Milk	89, 104
	Sour Milk	111, 101

Table notes: See Figure 1 for structures. Data are provided for each of two samples fortified at 0.01 ppm.

Registrant claimed a limit of detection for the method of 0.01 ppm, since this was the lowest level analyzed. Representative chromatograms were provided. The recoveries reported in Table 14 represent acceptable ranges.

The document MRID 42547121 describes validation of method AG-496A by the independent laboratory Cyal, Inc., Morrisville, NC. The independent laboratory reported that the method was run without communicating with registrant or any other lab that has run the method. A sample set of seven samples took one chemist less than 24 h to extract and analyze. The method was followed with only minor deviations. Representative chromatograms were provided. Milk samples were fortified at 0.01 and 0.05 ppm of each compound; Table 15 summarizes the recoveries of samples fortified at 0.01 ppm:

Table 15. Independent Laboratory Validation of Method AG-496A.

Compound	Sample	Recoveries, %
Atrazine	Fresh Milk	92, 116
	Sour Milk	84, 110
G-30033	Fresh Milk	114, 106
	Sour Milk	96, 117
G-28279	Fresh Milk	106, 120
	Sour Milk	100, 124
G-28273	Fresh Milk	106, 104
	Sour Milk	100, 120

Table notes: See Figure 1 for structures. Data are provided for each of two samples fortified at 0.01 ppm.

The recoveries indicated in Table 15 represent acceptable ranges. The independent laboratory concluded that the limit of determination was 0.01 ppm, the lowest level analyzed.

Conclusion 4c: Validation by registrant and independent laboratory validation of Method AG-496A using fortified samples were acceptable. The limit of determination is 0.01 ppm, the lowest level analyzed, for each of parent atrazine, G-30033, G-28273, and G-28229. The reregistration data requirement for independent laboratory validation of Methods AG-436 and AG-496 (both replaced by Method AG-496A) for milk is fulfilled. CBRS defers referral of this method for validation by an Agency laboratory until results of the hydroxyatrazine carcinogenicity study are reviewed and accepted.

The document MRID 42547122 contains a description of Method AG-593, which registrant indicates is a modification of Method EN-CAS 86-264. With this method, poultry lean meat, liver, and eggs are homogenized twice in acetone:water (1:1). The solid matrix is removed after each blending by centrifugation and the supernatant decanted into a flask. All traces of acetone are removed by evaporation. Peritoneal fat is extracted with hexane:acetonitrile (4:1), partitioned into an acetonitrile:water phase, and all traces of acetonitrile are removed by evaporation. One g of solid NaCl is then added to the remaining aqueous portions from either extraction procedure. Extracts are then diluted to 20 ml with a saturated NaCl solution, and loaded onto an Extrelut column. The column is eluted with 50% ethyl acetate:hexane for extracts from lean meat and eggs, or with 20% ethyl acetate:hexane followed by 50% ethyl acetate:hexane for extracts from fat and liver. Each fraction is collected separately. Fractions are reduced to dryness, reconstituted in

methanol:toluene (4:96), and analyzed by capillary (DB-17) gas chromatography with nitrogen-phosphorus detection. The detected compounds migrate as individual GC peaks, and residues are quantitated based on peak heights compared with standards.

Registrant Ciba-Geigy provided recovery data for poultry tissue samples fortified at 0.01, 0.05, and 0.20 ppm for each compound. Table 16 summarizes recovery data for the two samples for each compound fortified at 0.01 ppm:

Table 16. Recoveries from Fortified Samples with Method AG-593.

Compound	Sample	Recoveries, %
Atrazine	Liver	67, 72
	Lean Meat	75, 84
	Peritoneal Fat	73, 68
	Eggs	78, 77
G-30033	Liver	96, 101
	Lean Meat	73, 80
	Peritoneal Fat	85, 81
	Eggs	104, 106
G-28279	Liver	80, 86
	Lean Meat	76, 80
	Peritoneal Fat	86, 83
	Eggs	101, 97
G-28273	Liver	127, 113
	Lean Meat	58, 77
	Peritoneal Fat	88, 107
	Eggs	105, 85

Table notes: See Figure 1 for structures. Data are provided for each of two samples fortified at 0.01 ppm.

Registrant claimed a limit of determination for the method of 0.01 ppm, since this was the lowest level analyzed. Representative chromatograms were provided. Considering that they were obtained at the limit of determination, the recoveries indicated in Table 16 represent acceptable ranges.

The document MRID 42547123 describes validation of method AG-593 by the independent laboratory Cyal, Inc., Morrisville, NC. The

independent laboratory reported that recoveries were marginal in some tissues after the first trial. The method was discussed with registrant after the first trial, but no additional information was required for the second trial. A sample set of seven to nine samples took one chemist less than 24 h to extract and analyze. The method was followed with only minor deviations. Representative chromatograms were provided. Samples were fortified at 0.01 and 0.05 ppm of each compound, with additional liver samples fortified at 0.25 ppm. Table 17 summarizes recoveries from samples fortified at 0.01 ppm.

Table 17. Independent Laboratory Validation of Method AG-593.

Compound	Sample	Recoveries, %
Atrazine	Liver	71, 68
	Lean Meat	95, 105
	Peritoneal Fat	89, 80
	Eggs	102, 97
G-30033	Liver	119, 106
	Lean Meat	111, 114
	Peritoneal Fat	115, 136
	Eggs	98, 116
G-28279	Liver	103, 94
	Lean Meat	114, 97
	Peritoneal Fat	84, 86
	Eggs	95, 111
G-28273	Liver	118, 97
	Lean Meat	121, 98
	Peritoneal Fat	95, 113
	Eggs	89, 92

Table notes: See Figure 1 for structures. Data are provided for each of two samples fortified at 0.01 ppm.

The performing laboratory concluded that the limit of determination was 0.01 ppm, the lowest level analyzed. Considering that they were obtained at this limit of determination, the recoveries in Table 17 represent acceptable ranges.

Conclusion 4d: Validation by registrant and independent laboratory validation of Method AG-593 using fortified samples were acceptable. The limit of determination is 0.01 ppm, the lowest level analyzed, for each of parent atrazine, G-30033, G-28273, and G-28229. The reregistration data requirement for independent laboratory validation of Method EN-CAS 86-284 (replaced by Method AG-593) using eggs and poultry tissues is fulfilled. CBRS defers referral of this method for validation by an Agency laboratory until results of the hydroxyatrazine carcinogenicity study are reviewed and accepted.

Recommendation: In accordance with Conclusions 4c and 4d, the present submission fulfilled some reregistration requirements for analytical methods.

MAGNITUDE OF THE RESIDUE

The present submission also included the document:

Atrazine: Magnitude of the Residues in Corn Samples Following Application of AAtrex Nine-O, Ciba-Geigy Corporation, Project ABR-92028, October 27, 1992 (MRID 42547117).

This document reports the results of residue field trials on corn. AAtrex Nine-O (EPA Reg. No. 100-585), formulation 90WG, was applied to fields of corn at the 12 in stage at rates of 3, 6, 9, and 15 lb ai/A, representing 1X, 2X, 3X, and 5X, respectively, in a volume of 10 gal of tank mix per A. Registrant states that since the time the study was initiated, the maximum label rate for atrazine on corn has been reduced to 2.5 lb ai/A. Field trials were conducted in the midwestern states of IA, IL, IN, MN, and NE. Samples were taken of 30-day forage, silage forage, mature fodder, and mature grain. Samples were analyzed by Method AG-484 for residues of parent and the chloro metabolites G-30033, G-28279, and G-28273; by Method AG-571 for the hydroxy metabolite G-34048, and by Method AG-572 for the hydroxy metabolite GS-17794. Methods AG-571 and AG-572 have been combined and modified as Method AG-596, to detect both hydroxy metabolites with one method.

It appears that no fortified untreated samples were collected as concurrent samples for the purposes of demonstrating storage stability. Field samples were stored frozen for up to 26 mo before analysis for parent and the chloro metabolites, and up to 34 mo before analysis for the hydroxy metabolites. Registrant referred to storage stability data previously submitted which indicate that parent atrazine and other chloro triazines are stable under frozen storage for up to 25 mo, and noted that a study is in progress to determine the storage stability of residues of the hydroxy metabolites G-34048 and GS-17794. Registrant indicated that grain samples harvested from field

trials in NE at the 5X rate have been processed; residues are now being analyzed, and results will be provided in the future.

In grain samples from the 1X treatments, none of the marker residues analyzed was detected. Limits of detection were ≤0.01 ppm for atrazine and each of the chloro metabolites, and ≤0.02 ppm for each of the two hydroxy metabolites, for a combined limit of detection of 0.08 ppm. No detectable residues of parent and the chloro metabolites were found in grain samples treated at the 5X rate; in one of two trials at 5X, the hydroxy metabolites G-34048 and GS-17794 were detected at 0.03-0.04 ppm in grain.

In 30-day forage samples from the 1X treatments, parent atrazine residues were ≤0.05 to 0.61 ppm; G-30033, G-28279, and G-28273 were each nondetectable, ≤0.05 ppm, in all 1X forage samples. In 30-day forage samples treated at 1X, G-34048 residues were ≤0.02-0.32 ppm, and GS-17794 residues were ≤0.02-0.14 ppm.

In silage forage samples from the 1X treatments, atrazine was detected at ≤0.05-0.42 ppm. G-28273 residues in these samples were ≤0.05-0.12 ppm; no residues of G-30033 or G-28279 were detected in these samples. In silage forage samples, residues of G-34048 were ≤0.02-0.13 ppm, and residues of GS-17794 were ≤0.02-0.14 ppm.

In corn fodder samples from the 1X treatments, no atrazine or chloro metabolites were found, at a combined limit of detection of 0.20 ppm. G-34048 residues in these samples were ≤0.02-0.14 ppm, and GS-17794 residues were ≤0.02-0.24 ppm. In general, residues for all commodities were higher at exaggerated treatment rates.

CBRS Comments, Magnitude of the Residue

As indicated above, previous review noted that the current risk assessment for atrazine from corn grain shows a cancer risk of 8.4×10^{-6}, assuming anticipated residues of 0.1 ppm (CBRS No. 9167, 1/22/92, M.S. Metzger). For estimated risk to be less than 10^{-6}, residues on corn grain would have to be 0.012 ppm or less. For the field trial residue data presented, the combined limits of detection of six marker compounds are 0.08 ppm, nearly an order of magnitude greater. Thus, the fact that combined residues on grain are nondetectable adds little to current risk assessment. As discussion above indicates, the HED Metabolism Committee has taken the position that exposure assessment for atrazine should be conducted on the basis of the total radioactive residue (Conclusion 1e).

It appears that concurrent samples to demonstrate storage stability were not generated for these field trials. Registrant refers to a study in progress on storage stability of the hydroxy metabolites G-34048 and GS-17794, and refers to previously

submitted data to support storage stability of atrazine and the chloro metabolites.

Conclusion 5: With non-radiolabeled field trials in the midwest, residues of six marker compounds in or on corn grain were nondetectable at application rates of 1X. The combined limit of detection for the marker compounds was 0.08 ppm for corn grain. The residue data provided do not alter the conclusion that exposure assessment should be conducted on the basis of the total ^{14}C-labeled residue (Conclusion 1e). In addition, storage stability studies to support data on two of the marker compounds are in progress. CBRS declines review of the magnitude of the residue studies in corn, until results of the hydroxyatrazine carcinogenicity study are reviewed and accepted.

cc:Circ, Abbotts, Deschamp (for contractor), RF,
Atrazine List A File, SF
RDI:FBSuhre:5/24/93:MSMetzger:5/27/93:EZager:5/27/93
H7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:6/2/93
[redacted]JA6:atrazine.2