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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Memorandum

Subject: Atrazine Special Review. Metabolism, Revised Anticipated Residues.
MRID Nos. 412098-00, -01, -02, -03, -04, -05, -06, -07, -08.
DEB No. 5783

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Introduction

DEB met with representatives of Ciba-Geigy on 1/26/89 to discuss plant and animal metabolism data requirements for the atrazine Special Review. Ciba-Geigy representatives provided a summary of atrazine metabolism at this meeting and agreed to submit two documents, summaries of atrazine plant and animal metabolism data. The company stated that, based on these data, the Agency could determine that the hydroxymetabolites of atrazine need not be included in the total toxic residue for the pesticide. Nine documents have been submitted (MRID Nos. 412098-00 to -08) and 8 of these are reviewed here. This review discusses the metabolism of atrazine and includes the information in the documents provided with this submission (including separate reviews of studies not previously submitted) as well as studies previously submitted and included in the review of M. Metzger (9/14/88) which are related to atrazine metabolism.

The document not thoroughly reviewed here is MRID No. 412098-05 (ABR-89067) titled, Estimated Dietary Exposure of Hydroxyatrazine Metabolites to Man. This study included a Tolerance Assessment

System analysis of dietary exposure. We, therefore, refer this study to DRES/SACB for review. However, we do point out problems with the anticipated residues used in this TAS run. A single value of 0.1 ppm is used for all commodities including macadamia nuts, pineapples, corn, sorghum, wheat, millet, sugarcane, and animal products (except milk and eggs = 0.01 ppm). This value was translated to all crops from the atrazine residue (total ^{14}C) found in corn grain (treated preemergence with uniformly ring labeled ^{14}C atrazine at 3.0 lbs.a.i./A) of 0.07 ppm. Because the value is rounded up from 0.07 ppm to 0.1 ppm, and because this represents the total activity in corn grain, the submitter states that this is a worst case dietary exposure assessment. We note the potential for residues higher than 0.1 ppm of the hydroxymetabolites in pineapples and sugarcane, although metabolism data are not available to allow estimations of these levels. Additionally, secondary residues in animal tissues resulting from ingestion of biosynthesized residues (e.g. corn) treated post-emergence could be significantly greater since plant commodities treated post-emergence have a higher percentage of organosoluble metabolites which transfer to tissue more than the water soluble metabolites.

Conclusions

(1a) For the purposes of the atrazine Special Review, we consider the metabolism of atrazine in corn and sorghum to be adequately understood. In corn and sorghum atrazine is absorbed and translocated throughout the plant. The nature and composition of the residue depends on the length of time since treatment, and on the type of treatment (post-emergence vs. preemergence). In general, atrazine metabolism involves N-dealkylation, hydroxylation, conjugation with endogenous plant components (primarily glutathione, to a lesser extent lanthionine and mercapturic acid, and possibly to sugars), oxidation of the ring amines, and through combinations of these processes. At plant maturity, the total residue is composed of numerous metabolites: a small percentage of chlorometabolites (generally <8% for preemergence, ca. 12% for postemergence treatments), many water-soluble metabolites including hydroxymetabolites (some water-soluble metabolites comprising a significant portion of the total triazine residue were not identified), and bound residues which are released under mildly acidic conditions to produce water-soluble metabolites which were not identified. The majority of the total residue is composed of metabolites which contain the intact triazine ring, considered to be of toxicological concern. No single metabolite has consistently been shown to comprise the majority of the total residue at plant maturity in corn and sorghum (see the Appendix for a list of metabolites tentatively identified in these commodities).

(1b) Adequate metabolism studies have been submitted only for corn and sorghum. Some information is available in the literature suggesting that the composition, quantity, and nature of the residue varies among plants. Since the levels of secondary residues in animal tissues depends on the nature and quantity of the residues consumed, additional metabolism data for pineapples and sugarcane would be necessary to determine anticipated residues in milk for "local milk shed" diets which contain these feed commodities. Additional metabolism data would similarly be necessary for wheat and millet if the percent crop treated were to increase significantly for these commodities (currently both <1% crop treated).

(2) As part of the atrazine Special Review, DEB previously indicated that additional field trial data would be required for all commodities for which atrazine is registered, and that these data should include determination of residues of the hydroxymetabolites G-34048, GS-17794, GS-17792, and GS-17791 (M. Metzger, 7/12/88). Since all metabolites containing the intact triazine ring are now considered to be of toxicological concern, this data requirement should be revised such that all metabolites which contain the intact triazine ring are determined in these field trials. This could be accomplished by conversion of all metabolites containing the intact triazine ring to a single, quantifiable compound.

(3a) Based on the plant metabolism data submitted, we modify our previously calculated anticipated residues for corn forage, fodder, and silage to 5.0 ppm, 3.0 ppm, and 3.0 ppm respectively to reflect total triazine residues in these commodities (previous anticipated residues were 0.26 ppm for forage, 0.17 ppm for fodder, and 0.14 ppm for silage). Anticipated residues for other corn and sorghum commodities should not be changed (see M. Metzger, 9/14/88).

(3b) Insufficient data are available to modify our previous anticipated residue estimates for commodities other than corn and sorghum. However, it is likely that inclusion of all metabolites containing the intact triazine ring in the total toxic residue, rather than only parent plus chlorometabolites, would increase the anticipated residue estimates.

(4) For the purposes of the atrazine Special Review, the metabolism of atrazine in animals is considered adequately understood. The available animal metabolism studies indicate that secondary residues occur in eggs, milk, and

animal tissues as a result of animal ingestion of atrazine or its chlorometabolite G-28273. Much lower, but finite, residues occur in animal commodities as a result of animal ingestion of hydroxyatrazine, or corn and sorghum biosynthesized metabolites (^{14}C atrazine treated corn and sorghum (preemergence) fed to animals at plant maturity). The metabolism of atrazine or its chlorometabolite G-28273 in animals involves two major pathways: N-dealkylation, and conjugation with glutathione followed by modification of the glutathione moiety (see the Appendix for structures of tentatively identified metabolites). Since many metabolites remain unidentified or their identification is not certain, other minor processes may also occur. Modification of the glutathione moiety may include hydrolysis of amide bonds or cleavage of other bonds to form the cysteine or mercapturic acid conjugates, hydrolysis of C-S bonds to form sulfhydryl metabolites, oxidation of the sulfide to sulfoxide and sulfone, formation of methylthio derivatives, formation of disulfides (two triazine rings), and combinations of these processes. Chlorometabolites or other organosoluble metabolites account for a small portion of the total triazine residue (<7%) except in milk where the di-N-dealkylated metabolite G-28273 accounts for approximately 30% of the total residue. The majority of the residue is composed of numerous aqueous-soluble metabolites or non-extractable metabolites. In tissues, the non-extractable residue is released by treatment with collagenase or hypotonic solution indicating that the residue is not chemically bound, but is simply occluded in the sample matrix. In most cases, the total triazine residue is composed of numerous metabolites, each making up only a small proportion of the entire residue. Exceptions to this are milk, as mentioned, and ruminant liver where the cysteine conjugate of G-30033 accounts for approximately 25% of the total residue. The metabolism of hydroxyatrazine and biosynthesized metabolites in animals is therefore not well described.

(5) Based on the available animal metabolism data, we have recalculated anticipated residues for eggs, milk, and animal tissues to reflect the increased animal dietary burden which includes all metabolites containing an intact triazine ring, and the decreased transfer of residues to animal tissues by biosynthesized metabolites relative to chlorometabolites. These are listed below. See the discussion under "Detailed Considerations" for a more detailed discussion of these changes.

Commodity	Anticipated Residues (ppm) Old	New
Milk, national dairy cattle diet.....	0.0003	0.004
Milk, "local milk shed" dairy cattle diet.....	0.004	-
Meat, fat and meat by-products (except liver and kidney) of cattle, goats, hogs, horses and sheep	0.001	0.004
Liver of cattle, goats, hogs, horses, and sheep.	0.002	0.02
Kidney of "	0.001	0.006
Meat, fat, and meat by-products (except liver) of poultry.....	0	0.0006
Liver of poultry.....	0	0.002
Eggs.....	0	0.01
Egg whites.....	0	0.009
Egg yolks.....	0	0.01

Recommendations

DEB recommends that the Data Call-In for field trial data be modified as described in conclusion (2). We recommend that the revised anticipated residues given in conclusions (3) and (5) above be provided to DRES/SACB for a revised dietary risk assessment which is more reflective of dietary exposure to all metabolites of atrazine containing an intact triazine ring. Finally, we recommend that MRID No. 412098-05 be referred to DRES/SACB for review and comment.

Detailed Considerations

Plant Metabolism

Ciba-Geigy has submitted Atrazine: Nature of the Atrazine Residue in Plants (MRID No. 412098-01) which contains a summary section as well as 5 selected plant metabolism studies (GAAC-72022, GAAC-72081, ABR-79001, GAAC-73045, and ABR-87093). The first three of these were reviewed in the Residue Chemistry Chapter of the Atrazine Registration Standard Second Round Review (SRR). The final two studies are summarized later in this review.

A summary of the total radioactivity in various plant fractions from all available metabolism studies in which these data were obtained and reported is shown in Table 1.

Table 1: Distribution of the Total Radioactivity in Plants Treated with ^{14}C Atrazine or ^{14}C Hydroxyatrazine

Study	Commodity[1]	App. Rate[2] (lbs.a.i./A)	Total Radioactivity (ppm) Stalks	Hulls/ Cobs	Grain
GAAC-72081	sorghum (F)	3.0 (pre-E)	1.2	0.3	0.02
	" (GH)	2.5 (pre-E)	8.9	4.3	0.3
GAAC-72138	(F)	2.1 (post-E)	1.64		0.018
GAAC-75068/ GAAC-72128[3]	(F)	3.0 (3 apps.) (pre-E)	1.46 1.04		0.2 (year 2) 0.36 (year 3)
ABR-79001	corn (F)	3.0 (pre-E)	2.6	0.13	0.05
ABR-87093	(GH)	2.0 (pre-E)	5.29	0.45	0.30
GAAC-71022	(F)	3.0 (pre-E)	0.76	0.18	0.03
	(GH)	4.0 (pre-E)	6.8	0.6	0.06
GAAC-72122	(F)	4.0 (post-E)	5.42	0.25	0.07
GAAC-75083R/ GAAC-76011R	(GH)	3.0 (pre-E)	4.4	0.47	0.11
ABR-79087	(F)	3.0 (pre-E)	0.86	0.16	0.05
GAAC-73045[4]	(GH)	3.0 (pre-E)	0.98	-	-

[1]GH = greenhouse-grown, F = field-grown
[2]post-E = post-emergence application, pre-E = preemergence app.
[3]applications made at 3.0 lbs.a.i./A for 3 years; samples obtained for years 2 and 3
[4] ^{14}C hydroxyatrazine applied to corn

These data indicate that both atrazine and hydroxyatrazine (a major soil metabolite) are absorbed by the roots and rapidly translocated throughout the plant. Study GAAC-72122 indicates that absorption may also occur through leaves since greater than 20% of the total applied ^{14}C (foliar application) was aqueous extractable or non-extractable 1 day following treatment.

Following treatment of corn or sorghum with ^{14}C atrazine, the percentage of organosoluble metabolites decreases while the percentages of aqueous extractable and non-extractable residues increase. Data on the relative percentages of organo-, aqueous, and non-extractable residues at plant maturity are provided in Table 2. Data provided with ABR-87093 indicate that 85-90% of the non-extractable metabolites are released with mild acid treatment (1.0N HCl, 1.5 hours), and that the released metabolites are polar.

Table 2: Extraction Characteristics of ^{14}C Atrazine-Treated Corn and Sorghum at Plant Maturity

		Approximate Percentage ^{14}C Activity (%)[1]								
		Stalks			Hulls/Cobs			Grain		
Study	Commod	Org	Aqu	Non	Org	Aqu	Non	Org	Aqu	Non
GAAC-71022	corn	12-22	35-66	12-53	7-36	56-81	7-12	0-21	64-78	14-22
GAAC-72122		12	27	62						
ABR-79001		5	60	33	4	66	22	<0.1	60	38
ABR-79087		6	(46)	48	3	(48-67)	(30-51)			
ABR-87093		0.5	70	19	<0.5	66	24	7	47	54
GAAC-72081	sorghum	2	73	25	9	61	30	2	50	49
		5	61	34	3	48	50	2	44	54
GAAC-75068/ GAAC-72128		5	59	28						
		6	42	45				2	47	55
GAAC-72138		4	56	36						

[1]Values in parentheses are estimates extracted from data summaries

The organosoluble metabolites of atrazine include parent, the two mono-N-dealkylated metabolites (G-28279 and G-30033), and the di-N-dealkylated metabolite (G-28273). In Report No. GAAC-72122 (MRID No. 00161854), unknown organosoluble metabolites were found to account for approximately 10-16% of the total applied radioactivity in corn following post-emergence applications at the maximum application rate of 4.0 lbs.a.i./A.

Aqueous and non-extractable residues account for most of the residues found at plant maturity in corn and sorghum for all application scenarios. The major water soluble metabolites which were found at plant maturity were hydroxyatrazine (G-34048) and the mono-N-dealkylated hydroxymetabolites (GS-17792 and GS-17794). The di-N-dealkylated hydroxymetabolite (GS-17791) was identified in some corn studies, and the di-hydroxy metabolite (GS-11957) was found in sorghum.

The major water soluble conjugate reported was the glutathione conjugate of atrazine. The glutathione conjugate was found to comprise a large portion of the total radioactivity in immature plants, but was found only in trace amounts at plant maturity. Results support that at least one mechanism of the production of hydroxymetabolites is through a glutathione conjugate intermediate. Glutathione conjugates of either atrazine or its dealkylated derivative may be present since these compounds are not resolved with the analytical techniques used in most studies. Other conjugates tentatively identified include those of lanthionine and mercapturic acid.

Results from study ABR-87093 indicate that non-extractable (bound) residues are released (85-90%) under mildly acidic conditions (1.0N HCl, 1.5 hours). Although the structures of the released metabolites could not be identified, it was determined that the metabolites were polar and contained the triazine ring. All studies report that the total residue was composed primarily of metabolites which contain the intact triazine ring.

In summary, the metabolism of atrazine in plants (corn and sorghum) involves N-dealkylation, hydroxylation, conjugation with endogenous plant components (primarily glutathione, to a lesser extent lanthionine and mercapturic acid, and possibly to sugars), oxidation of ring amines, and through combinations of these processes. At plant maturity in corn and sorghum, the total residue is composed of numerous metabolites: a small percentage (generally <8%) chlorometabolites, many water soluble metabolites including primarily hydroxymetabolites (although some water soluble metabolites comprising a significant portion of the residue were not identified), and bound residues which are released under mildly acidic conditions to produce water soluble metabolites which have not been identified. The majority of the total residue is composed of metabolites which contain the triazine ring. No single metabolite has been shown to consistently represent the majority of the total residue at plant maturity (in corn and sorghum).

TOX has concluded that any metabolite which contains the intact triazine ring is of toxicological concern. Based on the available metabolism data, we cannot identify all of the metabolites which contain the intact triazine ring. Furthermore, we cannot identify a marker compound whose percentage of the total residue is sufficiently consistent that an accurate determination can be made of the total residue containing the triazine ring based on the marker. Therefore, based on the plant metabolism summary and on the data available on metabolism in DEB files, it is evident that in order to determine the total toxic residue, all components containing the intact triazine ring must be converted to a compound(s) of known structure which can be analyzed (possibly cyanuric acid) (we note that the analytical method used must have a sufficiently low limit of detection that an accurate and unambiguous determination may be made that the risk resulting from human ingestion of all commodities containing atrazine residues is acceptable). Therefore, considering the data provided in this submission, and the conclusion by TOX that all metabolites containing the intact ring are of toxicological concern, we modify our previous conclusion that residues of parent plus chlorometabolites and hydroxymetabolites are of concern. Rather, all metabolites containing the intact triazine ring are of concern. Additional field trial data must be provided for all commodities to which atrazine may be applied. These field trials must reflect all registered uses of atrazine

on these commodities, and must reflect the total residue including all metabolites which contain the triazine ring.

We note that registrant-conducted metabolism studies have been provided only for corn and sorghum. Studies are available in the literature for a variety of other commodities which suggest that some metabolic pathways, such as hydroxylation, do not occur in some plants. Therefore, the composition of the terminal residue will be different for different plants (even for different corn hybrids). The data available in the literature are not sufficient to show that the available residue data are sufficient for any particular commodity, or that the use of a marker compound is suitable for any commodity. Therefore, the field trial data discussed above apply to all crops for which atrazine is registered for application.

Anticipated Residues In Plant Commodities

DEB agreed to reevaluate previously determined anticipated residues based on the data submitted in this metabolism summary (see M. Metzger, 9/14/88). Adequate metabolism data were submitted only for sorghum and corn.

The previously determined anticipated residues for corn grain and forage were 0.10 ppm and 0.26 ppm respectively (M. Metzger, 9/14/88). Field and greenhouse metabolism studies in corn grain show total radioactivity ranging from 0.03-0.07 ppm (field) and 0.06-0.30 ppm (greenhouse). For corn stalks the total radioactivity ranged from 0.76-5.42 ppm (field) and 0.98-6.8 ppm (greenhouse). Based on these data, we maintain our anticipated residue estimate of 0.10 ppm for corn grain. For corn forage, we must consider the total triazine residue rather than just the residue of parent plus chlorometabolites. We, therefore, revise our anticipated residue estimate of 0.26 ppm for corn forage to 5.0 ppm, and proportionally modify the anticipated residues for corn fodder and silage to 3.0 ppm.

Our previous anticipated residue estimates for sorghum grain and forage were 0.13 ppm and 2.02 ppm respectively. based on the metabolism data shown in Table 1, we conclude that these anticipated residues are appropriate.

Modification of these anticipated residues may be necessary when the additional field trial data for corn and sorghum are submitted.

Animal Metabolism

Atrazine is an herbicide registered for use on plants which can be used as animal feeds. The nature and quantity of secondary residues in animal tissues, eggs, and milk depend on which components of the total toxic residue the animal consumes. The

major animal feeds to which atrazine may be applied are corn and sorghum, although sugarcane, pineapples, grass, and wheat (<1% crop treated for wheat) may also be treated. Adequate metabolism studies are available to determine the terminal residue (residue fed to animals) in corn and sorghum, but metabolism data are not available for the other commodities. Therefore, we discuss below the metabolism of atrazine, or its corn or sorghum biosynthesized metabolites, in animals. Since atrazine (or G-28273, an organosoluble chlorometabolite) and biosynthesized metabolites (primarily water soluble, non-extractable, or water soluble following mild acid treatment) likely represent the extremes in quantity of residue deposition in animal tissues and range of classes of metabolites, secondary residues in animal tissues resulting from animal consumption of treated sugarcane, pineapple, grass, and wheat will fall within this range. However, since the secondary residues in animal commodities depend so strongly on the nature of the terminal residue in plants, additionally metabolism data may be required for these commodities.

Numerous studies have been submitted in which cows or goats were dosed with ^{14}C atrazine for 10 days. Total recovery of radioactivity in these studies ranges from approximately 70-95%. Balance studies indicate that most of the ingested radioactivity is excreted in urine and feces with small percentages found in muscle, milk, and blood (1-3%), and smaller percentages in liver, kidney and fat (<1%). The residue levels found in tissues and milk generally follow the order liver>kidney>muscle>milk>fat (see Table 3). A small portion of the ingested radioactivity is recovered as $^{14}CO_2$ (<3%) indicating that triazine ring cleavage occurs to a small degree.

In tissues, residues of organosoluble, aqueous soluble, and non-extractable residues generally range from approximately <1-7%, 10-50%, and 55-81% respectively. Upon treatment in a hypotonic solution, or with a non-specific protease or collagenase, most of the non-extractable residue is released as water soluble residue. Since collagenase is capable of releasing non-extractable residues, these residues are likely to be simply occluded in the sample matrix rather than bound to any cellular constituents. Residues of organosoluble, aqueous soluble, or non-extractable residues in milk range from 30-60%, 20-40%, and 0-40% respectively, and in eggs from 0-15%, 30-80%, and 10-50% respectively.

Table 3: Total Radioactive Residues in Animal Tissues, Milk and Eggs Following Dosing with ^{14}C Atrazine, ^{14}C Hydroxyatrazine (G-34048), ^{14}C G-28273, or ^{14}C Biosynthesized Metabolites (Corn or Sorghum)

Animal	^{14}C Chemical	Dose (ppm Feed)	Residue (Total ^{14}C Activity, ppm) Liver	Kidney	Meat	Fat	Heart	Milk	Eggs[1]
Cows	atrazine	28	3.58		1.09	0.26		0.67	
		6.8	0.87		0.24	0.15		0.12	
		0.62	0.11		0.02	0.01		0.01	
	G-34048	0.62	0.007	0.004	0.0006	<00005	0.0008	0.003	
Goats	atrazine	44	4.6	3.45	1.13		1.05	0.70	
		33	5.16	3.32	0.95	0.10		0.63	
		27.2	3.03	1.76	0.54	0.06	0.54	0.89	
		5	1.26	0.81	0.13	0.06	0.15	0.16	
	atrazine + simazine	2.5 + 2.5	1.29	0.69	0.14	0.12	0.14	0.09	
	G-28273	5.8	1.3	0.98	0.34	0.09		0.172	
	biosynth[2]	0.95	0.01	0.003	0.0008	0.0008	<0.0006	0.004	
	biosynth[3]	0.012	0.0006	<0.0006	<0.0006	<0.0006	0.0006	0.0001	
	biosynth[4]	1.47	0.068	0.015	0.002	<0.001	0.003	0.003	
	biosynth[5]	0.32	0.036	0.01	<0.006	<0.006	<0.006	0.003	
Chick	atrazine	58	3.32	4.62	2.76	1.77	2.4		1.4/2.6
		50	3.15		3.40				1.15/2.5
	G-28273	5	0.55	0.90	0.50	0.04			0.21/0.36
	biosynth[7]	0.047	0.013	0.009	ND[7]	ND[7]			0.008/0.01

[1]Egg residues given as whites/yolks
[2]Goats were fed corn silage prepared from corn grown in soil treated at 3.0 lbs.a.i./A ^{14}C atrazine
[3]Goats were fed corn grain prepared from corn grown in soil treated at 3.0 lbs.a.i/A ^{14}C atrazine
[4]Goat was fed milo sorghum fodder treated preemergence with ^{14}C atrazine at 2.5 lbs.a.i./A in a greenhouse
[5]Goats were fed corn grain treated preemergence with ^{14}C atrazine at 3.0 lbs.a.i./A
[6]Chickens were fed corn grain treated preemergence with ^{14}C atrazine at 3.0 lbs.a.i./A
[7]ND = non-detectable residue, limit of detection not provided

Characterization of residues was accomplished using several techniques including ion exchange chromatography (anion and cation), one- and two-dimensional TLC, TLC-TLE, mass spectrometry, and other methods. In general, two major processes account for most of the metabolites identified: N-dealkylation, and conjugation of glutathione followed by modification of the glutathione moiety. Modifications to the glutathione moiety may include hydrolysis of the amide bonds and cleavage of other types of bonds to form the cysteine and mercapturic acid conjugates, hydrolysis of C-S bonds to form sulfhydryl metabolites, oxidation of the sulfide to sulfoxide and sulfone, formation of methylthio derivatives, formation of disulfides (containing two triazine rings), and combinations of these processes. Tentatively identified metabolites are shown in the Appendix. Although the structural assignments described in these studies are not adequately supported by the data in many cases, identification of these pathways as the major metabolic processes is demonstrated. Further characterization of these residues to more adequately demonstrate the validity of these structural assignments would not provide additional information necessary for Agency purposes.

In most samples examined, the total atrazine residue is composed of numerous metabolites, each of which accounts for only a small portion of the total residue. Exceptions to this are milk and liver. In milk, chlorotriazine residues comprise a large portion of the total residue (ca. 30% of the total residue, primarily G-28273). In liver samples, the cysteine conjugate of G-30033 was shown to comprise about 25% of the total residue.

When animals are fed ^{14}C hydroxyatrazine or biosynthesized metabolites, differences in residues are seen. When ^{14}C hydroxyatrazine was fed to a cow, secondary residues were found but were more than 10-fold lower in tissues, and 3-fold lower in milk, than when parent atrazine is fed at the same level. The nature of the residue resulting from feeding ^{14}C hydroxyatrazine was not characterized sufficiently because of low tissue residues. However, the data suggest that much of the residue in excrement is unchanged, while a smaller, but significant, portion of the residue undergoes N-dealkylation or other processes to form more basic residues. Residues in tissues were not characterized.

When corn or sorghum biosynthesized metabolites were fed to goats, residues in tissues and milk were lower than when parent atrazine was fed, but the factor by which these residues were lowered were variable depending on which tissues and which studies were compared. The differences in tissue residues were not as great as when comparing those resulting from feeding atrazine vs. hydroxyatrazine. The difference in tissue residues in chickens resulting from feeding ^{14}C atrazine or corn biosynthesized metabolites could not be accurately determined because of the large difference in feeding levels used in the studies (atrazine = 50 ppm or 58 ppm in feed, biosynthesized metabolites = 0.047 ppm in feed). Some modification of the

biosynthesized residue is seen for goats and chickens, but the tissue and excrement residues were not sufficiently characterized to determine the extent or nature of these modifications.

In summary, the available studies sufficiently describe the metabolism of atrazine in ruminants and poultry. When atrazine or its chlorometabolite G-28273 are fed to animals, most of the residue is excreted, while some residue is found in milk, eggs, and tissues. Tissue residues are composed primarily of numerous water soluble metabolites or non-extractable metabolites which are released as water-soluble metabolites by enzyme treatment. These residues result primarily from N-dealkylation, and conjugation with glutathione followed by modification of the glutathione moiety. Small percentages of the chlorometabolites are also found. The residue in milk is composed of a significantly larger percentage of chlorotriazine residues (30% G-28273). When hydroxyatrazine or biosynthesized metabolites (mostly aqueous soluble or non-extractable) are fed to ruminants, significantly lower tissue residues result than when parent or G-28273 are fed. These tissue residues have not been sufficiently characterized to allow the nature of the resulting residue to be determined.

TOX has recently concluded that any atrazine metabolite which contains the triazine ring is of toxicological concern. The available animal metabolism studies indicate that less than 3% of the residue ingested by animals is released as CO_2 (ring cleavage), and that the majority of the residue is composed of numerous metabolites , all of which contain the intact triazine ring. Since no single metabolite comprises a significant portion of the total residue except G-28273 in milk and the cysteine conjugate of G-30033 in liver, and since the percentages of these residues will vary depending on the specific animal and the metabolites consumed, a marker compound which would consistently indicate the total triazine residue in milk, eggs, and animal tissues is not available. Therefore, the total triazine residue may require quantification by conversion of all of the triazine metabolites to a common compound which can be quantified.

Anticipated Residues in Animal Commodities

The anticipated residues previously calculated for residues in animal commodities are shown in Table 4 together with revised anticipated residues. The differences between the new anticipated residues and those previously calculated result primarily from the increased residues in animal feed items since all metabolites containing a triazine ring are now considered, rather than just the chlorometabolites. Also, several additional potential animal diets are considered which represent possible diets which could typically be fed nationally. In general, while the decreased animal tissue deposition of residues resulting from feeding terminal atrazine residues to animals are lower than those resulting from feeding parent or other chloro-compounds, the increased levels of these terminal plant residues which are

consumed by animals lead to higher total atrazine residues in animal tissues, milk, and eggs.

For beef cattle, six additional potential diets were considered. Major components of these diets are shown in Table 5.

Table 4: Anticipated Residues of Atrazine and Its Metabolites in Animal Commodities - Previously Calculated and Revised Levels Reflecting All metabolites With an Intact Triazine Ring

Commodity	Anticipated Residue (ppm) Old	New
Milk, national dairy cattle diet	0.0003	0.004
Milk, local milk shed diet	0.004	-
Meat, fat and meat by-products (except liver and kidney) of cattle, goats, hogs, horses and sheep	0.001	0.004
Liver of cattle, goats, hogs, horses and sheep	0.002	0.02
Kidney of cattle, goats, hogs, horses and sheep	0.001	0.006
Meat, fat and meat by products (except liver) of poultry	0	0.0006
Liver of poultry	0	0.002
Eggs	0	0.01
Egg whites	0	0.009
Egg yolks	0	0.01

Table 5: Beef Cattle Diets

Feed Item	Anticipated Residue (ppm)[1]	Percent (%) in Diet 1	2	3	4	5	6
Corn silage	3.5	82.2	58.7	16.7	69.3	38.4	16.8
Corn grain	0.07	0	0	0	11	57.6	80.7
Soybean meal	0	2.7	0.7	0	3.1	1.6	0
Barley	0	13.7	40	80	0	0	0
Alfalfa	0	0	0	0	15.7	0	0
Other	0	1.4	0.6	3.3	0.9	2.4	2.5
Diet. Burden-->		2.9	2.1	0.58	2.4	1.4	0.64

[1]Percent crop treated values are incorporated into these anticipated residues

Two studies were used in conjunction with these animal dietary burdens to determine secondary residues in animal tissues: GAAC-

71021 (corn biosynthesized silage and grain metabolites fed to goats at 0.95 ppm and 0.012 ppm respectively, MRID No. 412098-06) and ABR-89054 (corn biosynthesized grain metabolites fed to goats at 0.32 ppm, MRID No. 412098-08). Results of these studies are shown in Table 3 at the beginning of this section.

For dairy cattle, four additional potential diets were considered (taken from Forages: The Science of Grassland Culture, Heath, Barnes, and Metcalfe, Eds., 1985). Major components of these diets are shown in Table 6. Three studies were used in conjunction with these animal dietary burdens to determine secondary residues in milk: GAAC-71049R (atrazine fed to goats at 0.62 ppm in their diets, MRID No. 404313-46) and GAAC-71021 (referenced above). Anticipated residues were calculated for milk using two methods. First, residues resulting in milk from ingestion of chlorometabolites only (for grass hay and wheat middlings)(GAAC-71049R) were calculated and then added to residues resulting in milk from feeding biosynthesized metabolites (from corn grain and silage)(GAAC-71021). Secondly, anticipated residues in grass hay and wheat middlings which represent chlorometabolites only, were assumed to account for 5% of the total triazine residue, since in most metabolism studies available (none for these commodities, however), chlorometabolites, or other organosoluble metabolites account for approximately 5% of the total triazine residue. Residues were then combined for grass hay and corn silage, and for wheat middlings and corn grain, and used in conjunction with the biosynthesized metabolites metabolism study (GAAC-71021) to determine anticipated residues in milk. Both of these methods gave similar results showing an anticipated residue in milk of 0.004 ppm.

Table 6: Dairy Cattle Diets

	Anticipated Residues % Crop treated Incorp.		Percent (%) in Diet			
Feed Item	Chloro's	Biosynth	1	2	3	4
Grass hay	0.09		0	10	20	30
Alfalfa	0	0	25	17	8	0
Corn silage		3.5	25	25	25	25
Oats	0	0	16	10	5	0
Wheat middlings	0.0002		10	7	3	0
Corn grain		0.07	15	18	21	23
Soybean meal	0	0	3	7	11	15
Linseed oil	0	0	5	5	5	5
Other	0	0	1	1	2	2

For broilers (poultry), the dietary burden was calculated using a poultry diet consisting of corn grain (65.2%, 0.07 ppm anticipated residue), soybean meal (26.4%, 0 ppm), and other feeds (8.4%, 0 ppm). For laying hens, the dietary burden was calculated using a poultry diet consisting of corn grain (61%,

0.07 ppm anticipated residue), milo sorghum grain (10%, 0.091 ppm), soybean meal (11.5%, 0 ppm), and other feeds (17.5%, 0 ppm)(poultry diets taken from Feeds and Nutrition, Complete, Esminger, M.E., & Olentine, C. G., 1978). These dietary burdens were used in combination with ABR-89006 (MRID No. 412098-07)(chickens fed ^{14}C atrazine corn biosynthesized metabolites at 0.047 ppm in their diets) to determine anticipated residues in eggs and chicken tissues..

Review of Individual Studies Not Previously Reviewed: Plant Metabolism

GAAC-73045 (MRID No. 412098-01)

Corn was grown in a greenhouse in buckets of soil which was previously treated with uniformly ring labeled ^{14}C hydroxyatrazine (G-34048) at a rate equivalent to 3.0 lbs.a.i./A. Tissue samples (presumably whole plant, but not stated) were obtained at 15 weeks and ground with dry ice followed by extraction with methanol:water (80:20), evaporation of the methanol, partitioning with hexane, and isolation of the residue from the resulting aqueous fraction. Following clean-up using a Dow 50W-X8 resin, a cation exchange column was used to isolate specific, weakly basic metabolites whose identification was the purpose of this study. One dimensional TLC (6 solvent systems), two-dimensional TLC (4 sets of solvent systems), and electrophoresis were used to identify metabolites by comparison with reference standards (reference standards were available only for G-28251, G-34048, GS-17794, GS-17792, GS-17791, and GS-11957). Mass spectra were also obtained.

Approximately 5% of the total radioactivity was organoextractable, 35% was non-extractable, and 64% was water soluble. The water soluble residue was chromatographed on the cation exchange column to obtain the 3 major water soluble peaks of interest in this study. TLC and TLE analysis of these peaks indicate that they have mobilities consistent with the metabolites GS-11957 (16.9% of the total radioactivity), GS-17794 (34.7%), and G-34048 (7.8%). The peak corresponding to GS-11957 was also found to contain GS-17792 (3%). Mass spectral data were inconclusive.

ABR-87093 (MRID No. 412098-01)

Corn (type unspecified) was treated with uniformly ring labeled ^{14}C atrazine in a greenhouse at a rate equivalent to 2.0 lbs.a.i./A. Corn was treated preemergent. Further information regarding application methods, rates, times, etc. were not provided. Corn was harvested at 4 weeks (stalks), 11 weeks (stalks), and 15 weeks (maturity, stalks, grain and cobs), and the samples were divided into two parts labeled "A" and "B". Parts A and B were combined for homogenation. Samples were combined and radioassayed using method AG-252. Samples

containing residues greater than 0.05 ppm were extracted using SOP 4.65. Radioassays were done using Method AG-276. Samples were stored frozen for an unspecified length of time (although the "testing period" for the "biological phase" is reported as 3/25/86 to 7/8/86, and for the analytical phase as 11/7/86 to 10/10/87, implying a 4-month minimum storage time). Polar metabolites were characterized by one- or two-dimensional TLC, ion exchange chromatography, and mass spectral analysis. For TLC analyses, standards were applied with the samples when appropriate, and radioautograms were prepared from the developed plates. Quantification was accomplished by scraping the appropriate zones from the TLC plates, and radioassay of the scraping using a scintillation counter. Ion exchange chromatography was carried out on both anion and cation exchange columns, and HPLC was employed to verify the identities of some metabolites. Mass spectral analyses were also performed to verify the identity of some metabolites. Sufficient raw data including chromatograms, histograms, photographs of TLC plates, and mass spectra were submitted.

The total radioactivity in the whole plant samples at 4 and 11 weeks were 5.48 ppm and 2.31 ppm respectively. At maturity the total radioactivity was 5.29 ppm in stalks, 0.30 ppm in grain, and 0.45 ppm in cobs. Most of the radioactivity in these samples was aqueous soluble (46.9 - 81.2%), with little organoextractable residue found (maximum 6.8% in grain at maturity). The percentage of non-extractable residue ranged from 13.1-23.9% (except in grain at maturity = 53.6%).

The presence of at least 15 aqueous extractable metabolites was demonstrated in this study. At maturity, at least 10 of these metabolites were shown to be present. The hydroxymetabolites (GS-17792, GS-17794, and G-34048) account for 41%, 12.5% and 30.5% of the total radioactivity in corn stalks, grain and cobs respectively (at maturity). These metabolites were identified by cochromatography with reference standards (HPLC, and 2-D TLC), and by their mass spectra. The other metabolites were not conclusively identified. They were tentatively identified as atrazine sugar conjugates or as conjugates of other endogenous compounds. the unidentified components account for 29.2%, 34.4% and 35.7% of the total radioactive residue in stalks, grain, and cobs respectively; and the individual unidentified components account for approximately 1.1-5.6%, 0.9-10.6% and 0.9-19.2% of the total radioactivity respectively.

The non-extractable portion of the residue was treated with 1.0 N NH_4OH (1.5 hours), 1.0 N HCl (1.5 hours), or cellulase (72 hours) releasing 30%, 85-90%, and 75-80% of the radioactivity respectively. The acid released residue was further characterized since acid treatment released a greater percentage of the residue than did base or enzyme treatment. Partitioning with ethyl acetate under acidic, basic, and neutral conditions showed no transfer of residue to the organic solvent indicating that the released residue was polar. Attempts to further

characterize the residue using TLC and cation exchange chromatography were unsuccessful.

Reviews of Individual Studies Not Previously Reviewed:
Animal Metabolism

ABR-89026/AbR-86056 (MRID No. 412098-03)

A lactating goat was administered ^{14}C atrazine in its diet for 10 days at a dose equivalent to approximately 33 ppm in the feed. Feces, urine and milk were collected daily, blood was collected every other day, and tissue samples were collected at sacrifice (24 hours after the last dose). Study No. ABR-89026 is an extension of ABR-86056 in which samples from the latter study were further characterized. Feces and tissue samples were homogenized (AG-223), and all samples were extracted (AG-214) using standard Ciba-Geigy methods. Residues were characterized using anion and cation exchange chromatography and TLC (one- and two-dimensional). A liver sample was treated with protease to release bound residues, and a urine sample was treated with glucuronidase to determine the presence or absence of glucuronide conjugates. Some samples were esterified and compared with esterified samples using TLC.

The results reported in ABR-86056 indicate that greater than 93% of the total radioactivity was excreted in the feces or urine. Tissue residues were as follows: leg muscle (0.95 ppm), tenderloin (0.95 ppm), kidney (3.32 ppm), omental fat (0.08 ppm), and back fat (0.10 ppm). Residues in milk plateaued by day 6 (maximum milk residue = 0.63 ppm), urine by day 7 (maximum urine residue = 9.5 ppm), and feces by day 6 (maximum feces residue = 7.64 ppm). Partitioning characteristics are presented in Table 7.

Table 7: Partitioning Characteristics of ^{14}C Atrazine Residue in Goat Tissues, Feces, Blood, and Milk

		Percent (%) in Extraction Fractions		
Sample	Residue (ppm)	Organic Soluble	Aqueous Soluble	Non-Extractable
Feces, day 7	6.83	6.74	27.56	55.11
Leg muscle	0.93	2.55	26.62	62.90
Liver	4.75	0.68	8.21	81.28
Liver after protease	4.75	0.68	70.32	29.00
Blood, day 8	0.87	2.70	18.71	76.04
Kidney	2.86	0.93	20.99	79.48
Milk, day 7	0.60	64.5	36.30	-

In tissue samples, only a small portion of the total residue is organosoluble, while in milk, most of the residue is

organosoluble, and the rest is aqueous soluble (no bound residues). In all samples except milk, most of the residue is non-extractable. Following protease digestion of liver samples, most of the non-extractable residue is released as aqueous soluble residue. TLC analysis of the milk, feces and urine samples shows no detectable residues of parent atrazine, small percentages of the mono-N-dealkylated metabolites (G-28279 and G-30033) in milk and urine (<1.5%), and larger percentages of the di-N-dealkylated metabolite (G-28273)(urine = 8.5%, feces = 5.4%, and milk = 45%). Results of the ion exchange chromatography analyses indicate the absence of glucuronide conjugates of atrazine in urine, and show the presence of a major neutral or acidic peak in addition to the peak for G-28273 in milk.

In study No. ABR-89026, residues in feces were further characterized from samples obtained in ABR-86056. The sample storage time was approximately 3 years. The submitter states that the residue is stable during this 3 year period based on comparison of 2-dimensional TLC plates and cation exchange chromatography histograms submitted. However, there are significant differences between the TLC plates and histograms provided indicating decomposition during storage. Furthermore, Report No. ABR-79001 reports that amino acid conjugates degrade in frozen corn plants when stored for greater than 6 months. Considering these problems, the results of these studies must be viewed cautiously.

Feces were extracted with 9:1 methanol:water with 28.8% of the residue extracted and 72.2% non-extractable. The methanol:water extract was further partitioned first with chloroform, and then the aqueous fraction with ethyl acetate, and cation exchange chromatography analysis (histograms) were obtained for the ethyl acetate, chloroform, and water extracts. Two-dimensional TLC was also performed for these samples, as well as for esterified glucuronidase treated, and sulfatase treated subsamples of each of these samples. Four separate peaks (obtained from the cation exchange column) for the water extract were isolated and analyzed by 2-D TLC, and subsamples were treated with glucuronidase, sulfatase, or were esterified and analyzed by 1-D TLC. Peak 1 from the cation exchange column was isolated and analyzed on an anion exchange column. The two major peaks from the anion exchange column were isolated and analyzed in a manner similar to the peaks isolated from the cation exchange column. We note that no characterization was attempted for the 72.2% of the residue that was not extracted by methanol:water.

The text of the study states that for all 2-D TLC analyses, solvent A (chloroform:methanol:formic acid:water, 70:25:4:2) was used followed by solvent B (n-butanol:glacial acetic acid:water, 133:33:33) in the second dimension. Later the text refers to R_f values in Table 1 (of the submission) in reference to the 2-D TLC in which R_f values are given for solvent A and solvent C (methyl ethyl ketone:acetonitrile:acetic acid:water, 3:4:1:2). We will assume that solvents A and B were used in the 2-D TLC

experiments, and that the references to R_f values in Table 1 were meant to address 1-D TLC analyses of some samples, or the first dimension of 2-D TLC analyses.

Analysis of the chloroform extract (of the methanol:water extract) indicate the presence of parent, the two mono-N-dealkylated metabolites (G-28279 and G-30033), the di-N-dealkylated metabolite (G-28273) and the methylthio derivative of the de-isopropylated metabolite (designated XV). The submitter states that the presence of a mono-N-dealkylated cysteine conjugate (XXV or XXIV) and the N-deethylated mercapturic acid conjugate (XXIX) are also demonstrated. However, the evidence for XXV or XXIV is inconclusive based only on comparison to an XXV standard on 2-D TLC using a system in which several other potential metabolites have similar migration. Regarding XXIX, the basis for this structural assignment is uncertain since no standards were run either in the TLC or the cation exchange chromatography experiments.

Analysis of the ethyl acetate extract (of the methanol:water extract) indicates the presence of G-28273 as the major component (ca. 70%). Other structural assignments are either unsupported by TLC or cation exchange chromatography runs using reference standards, or are tenuous considering the other possible metabolites which respond similarly under the TLC and chromatographic conditions used.

Analysis of the aqueous extract (of the methanol:water extract) revealed the presence of many water soluble metabolites. Identification of metabolites based on similar behavior in TLC systems and similar cation exchange chromatography elution volumes indicate that numerous metabolites may be present which result from N-dealkylation and/or glutathione or cysteine conjugation followed by further modification of the glutathione moiety. Although these metabolites may be present, this has not been rigorously shown since the behavior of the standards and unknown metabolites were similar but not identical, since more than one metabolite could account for the behaviors in the systems used in many cases, and because the behaviors of reference standards in the systems used were not provided for many metabolites tentatively identified.

In summary, the results of this study indicate that numerous metabolites result from oral administration of atrazine to goats. In all tissues and products except milk, most of the residue is non-extractable. In milk most of the residue is organoextractable and consists primarily of G-28273. Most non-extractable residue is converted to aqueous soluble residue using a protease enzyme. Metabolites which have been adequately identified in goat tissues or milk include parent atrazine, the mono-N-dealkylated metabolites (G-28279 and G-30033), the di-N-dealkylated metabolite (G-28273), and the methylthio derivative of the deisopropylated metabolite. Less conclusive evidence is presented for many other metabolites which result from N-

dealkylation, conjugation with cysteine or mercapturic acid, conjugation with glutathione followed by modification of the glutathione moiety, or a combination of these processes. The total residue of parent plus chlorometabolites (currently the only components included in the tolerance expressions), does not comprise more than 10% of the total residue (except in milk), usually less. Most of the residue (except in milk) is composed of numerous non-extractable metabolites (which are released as aqueous soluble metabolites in the presence of a protease enzyme), and aqueous extractable residues.

ABR-89027/ABR-86056 (MRID No. 412098-04)

As in the case of ABR-89026, ABR-89027 is a continuation of ABR-86056 in which samples of goat tissues and urine were further characterized after 3+ years frozen storage (see the review of 89026/86056 for background information). Residues were characterized by ion exchange chromatography (cation exchange column - Aminex A-4 resin; anion exchange columns - DEAE sephadex A-25 resin, strong anion exchange (SAX) Bond Elut cartridge), TLC (1- and 2-dimensional), chromatography using Amberlite XAD-4 resin, HPLC, and/or mass spectral analysis. Some samples were additionally characterized following enzyme treatment (collagenase, protease, glucuronidase, or sulfatase) or esterification.

Samples were stored for greater than 3 years between sampling and analysis. To demonstrate storage stability during this time, cation exchange chromatography histograms were submitted of day 7 urine (1986) and day 10 urine (1989). These histograms do not show that the residues are stable during this storage time because (a) they are of different samples, (b) there are differences between the histograms, and (c) only the aqueous fractions of the urine samples are presented. Additionally, residues in feces samples stored the same length of time (ABR-89026) did not appear to be stable. Therefore, any conclusions drawn from this study must consider the possible degradation during storage.

Urine samples were extracted with ethyl acetate. The ethyl acetate fraction accounted for 6% of the total radioactivity, and the aqueous fraction 94%. Assignment of structures to the TLC spots and peaks in the cation exchange chromatography histograms were made by comparison to standards. Although 10 structures were identified which behave similarly to standards in the systems used, other metabolite standards also behaved similarly in these systems. Therefore, the identification of these metabolites has not been conclusively shown. The metabolites tentatively identified include those resulting from the metabolic pathways of N-dealkylation and/or conjugation with glutathione followed by modification of the glutathione side chain.

TLC and cation exchange chromatography of the aqueous soluble residue showed numerous unresolved peaks. In order to resolve

and identify these peaks, the aqueous fraction was chromatographed on a sephadex A-25 DEAE preparative column giving 2 major peaks (A and B), and each of these two peaks was chromatographed using a preparative cation exchange column resulting in the isolation of 8 samples (A1-A6, B1-B2). Each of these 8 samples was further analyzed by 2-D TLC to determine the identity of the metabolites present.

Assignment of identities to unknown compounds was made based on (1) R_f values in 2-D TLC, (2) elution volume on a cation exchange column, (3) potential for esterification to form less polar compounds (indicating the presence of carboxylic acid groups), (4) susceptibility to digestion with a sulfatase which would cleave C-S linkages (sulfides, not sulfoxides or sulfones), and (5) susceptibility to cleavage by glucuronidase (the glucuronidase used was apparently also contaminated with sulfatase). Results of 2-D TLC and cation exchange chromatography of reference standards were not available for most proposed metabolite structures. Although in most cases the structural assignments made were consistent with the available data, insufficient information was presented to conclude that all of the proposed structures presented were correct. However, sufficient information is available to make the following conclusions regarding atrazine metabolites found in goat urine:

- N-dealkylation is a major metabolic pathway in goats
- conjugation of atrazine occurs through a sulfide linkage at position 6 (displacing chlorine) to form the glutathione conjugate of atrazine and numerous other related metabolites (including N-dealkyl conjugates and probably conjugates resulting from further degradation of the glutathione portion of the molecule)
- numerous metabolites are present in goat urine, most of which are water soluble, and many of which have not been thoroughly characterized
- no single metabolite comprises greater than about 20% of the total triazine residue, most much less

Liver samples extracted with ethyl acetate contained 0.7% organoextractable, 8.2% aqueous soluble, and 81.3 non-extractable residue. Treatment of the non-extractable residue with a non-specific protease, with collagenase, or with tyrodes solution (hypotonic) followed by homogenation, released 84%, 81%, and 54% of the bound residue respectively. However, comparison of cation exchange chromatography histograms of the released metabolites showed that treatment with the protease changed the components comprising the residue relative to non-enzymatic treatment (tyrodes solution) and collagenase treatment. Since treatment with collagenase released most of the bound residue and caused fewer changes in the composition of the released residue than did protease treatment, the collagenase treated samples were further

characterized. These results indicate also that most of the "bound" residue is simply associated with the liver cells rather than bound to any cellular component (because of the specificity of collagenase).

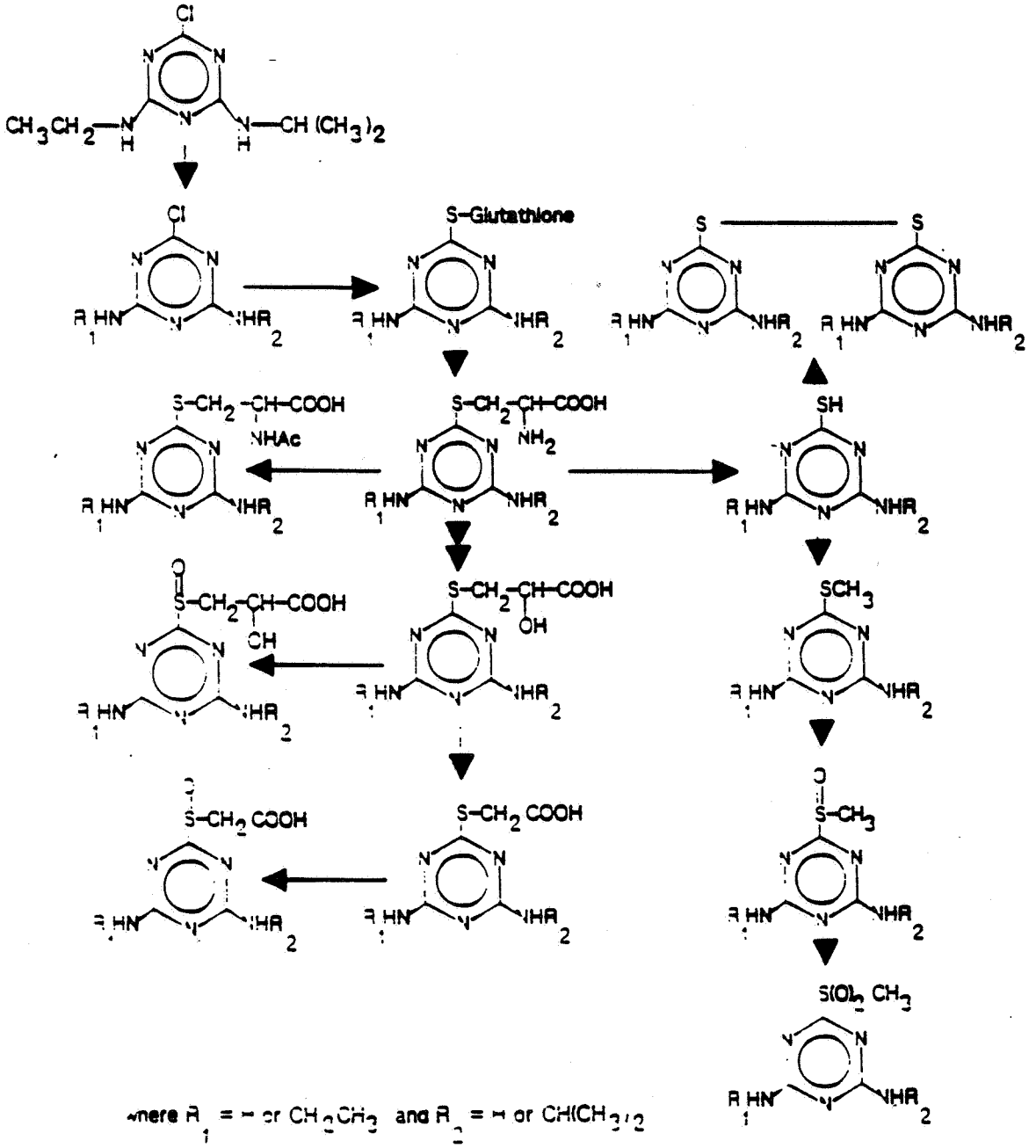
Residues in the liver filtrate were partitioned into methanol, methanol:water (50:50), and water fractions, with most of the residue found in the latter 2 fractions. The cysteine conjugate of the N-deethylated metabolite was shown to comprise from 25-30% of the total residue in liver (2-D TLC and mass spectrometry of the major component of the methanol water fraction). Several other metabolites were postulated to be present in the aqueous fraction, but identification of these metabolites were made solely on the elution volume from the cation exchange column since 2-D TLC analyses gave results which were not consistent with the proposed structures (9 structures: mercapturic acid and cysteine conjugates of atrazine and its 3 N-dealkylated metabolites, and the glutathione conjugate of G-28273).

Leg muscle and kidney tissue were treated with collagenase, and the filtrates were analyzed by cation exchange chromatography (both samples) or 2-D TLC (kidney only). Results of the analysis of residues in leg muscle indicate the presence of G-28273. The presence of the mercapturic acid conjugate and four other metabolites was also postulated (the mercapto-derivatives of G-28273, G-28279, and G-30033, and the sulfoxide derivative of G-28279). The structures of nine metabolites were tentatively identified in kidney tissues (G-28273, the mercapturic acid conjugates of G-28273, G-28279, and G-30033, the sulfoxide derivatives and the cysteine conjugates of G-28279 and G-30033, and the mercapto derivative of G-30033). As described in earlier parts of this study, structural characterization is ambiguous because of the large number of potential metabolites which behave similarly in the analytical systems used, and because results of the behaviors of many reference standards in these systems were not provided.

In summary, this study adequately demonstrates that goats dosed with atrazine metabolize the pesticide by N-dealkylation, conjugation with plant components through a sulfide linkage at position 6 of the ring, modification of the endogenous plant component attached by this bond, and by combinations of these processes. The presence of the glutathione conjugate of atrazine and the cysteine conjugate of G-30033 has been adequately shown. The presence of esterifiable moieties in the conjugates, probably carboxylic acid groups, has been demonstrated. Numerous metabolites were present in urine and animal tissues, most of these water soluble following treatment with collagenase. A small percentage of organosoluble residue was found. No single metabolite accounted for greater than approximately 10% of the total residue except in liver where the cysteine conjugate of G-30033 accounted for 25-30% of the total residue. The proposed pathway for metabolism of atrazine in animals shown in Figure 1 is consistent with the data and likely represents the major

metabolic pathways involved. However, because of the ambiguity in the characterization of these residues, and because some metabolites were not identified, the possibility of other metabolic pathways is not dismissed.

Figure 1: Proposed Pathway of Atrazine Metabolism in Animals



ABR-89065 (MRID No. 412098-06)

ABR-89065 is a review of four studies submitted with this report (GAAC-71047, -71021 parts I and II), -72088) which were not previously submitted, two volume accompanying this submission but reviewed separately (ABR-89026, -89027), and three studies previously submitted (GAAC-72131, -71049R, ABR-87064) and reviewed (M. Metzger, 9/14/88). These studies examine the differences in the amount and composition of the residue resulting from feeding animals parent atrazine, the di-N-dealkylated metabolite G-28273, hydroxyatrazine, or plant biosynthesized metabolites. The studies which have not been previously reviewed are reviewed separately in this memorandum. We note that only Ciba-Geigy reviews of these studies (GAAC-71047, -71021 (parts I and II), -72088) were submitted which contain insufficient information and raw data to allow a complete evaluation of the adequacy of the data.

The submitter concludes that "hydroxytriazines do not lead to tissue residues" but " pass through the animal unchanged". The submitter further concludes that the small amounts of chlorotriazines found in plants lead to the tissue residues seen in animals. DEB cannot fully concur with these conclusions.

Study GAAC-71047 (MRID No. 412098-06) shows that residues do result in milk and cow tissues as a result of feeding hydroxyatrazine (G-34048) to the cow, although these residues are lower than residues resulting from feeding atrazine or the chlorometabolite G-28273. These residues are greater than 10-fold lower in cow tissues, but only 3-fold lower in milk. This study also shows that a large portion of the residue excreted in the urine is unchanged G-34048 although the mono-N-dealkylated hydroxymetabolites are also present as well as a small percentage of unknowns. Residues in tissues and milk were not characterized.

Residues in animal tissues resulting from feeding biosynthesized metabolites (corn grain or fodder, or sorghum fodder) are also lower than residues resulting from feeding ^{14}C atrazine or ^{14}C G-28273, although the amount by which these residues are lower varies depending on which studies are compared and on which tissues are considered. Residues resulting in various animal tissues are, on average, 5-30 fold lower when biosynthesized metabolites are fed than when the chloro compounds are fed. Again, this difference is lower for milk than for tissues. Comparison of tissue residues can be made only for ruminants (goats, cattle) since the differences in the feeding levels in the chicken studies (5-58 ppn for chloro compounds, 0.047 ppm for biosynthesized metabolites) is too large to allow accurate comparisons to be made.

Comparison of the compositions of residues in ^{14}C atrazine treated feeds (biosynthesized metabolites) and animal tissues

(specific fractions of excreta and rumen) indicate that, to the degree to which the fractions compared are representative of the entire ^{14}C residue, biosynthesized metabolites are modified to some degree by the animal. In ruminants, the residue is modified to some extent, but the hydroxymetabolites (G-34048, GS-17792, GS-17794) still comprise a major portion of the residue. In chicken, the same is true but the residue appears to be modified to a greater extent. In both cases, it is likely that the residue is modified primarily by N-dealkylation, and to a lesser extent by other undetermined processes. The extent to which the entire residue is modified cannot be determined based on the available data.

GAAC-71047 (MRID No. 412098-06)

A lactating cow was fed uniformly ring labeled ^{14}C hydroxyatrazine (G-34048) in its diet for 10 days at a rate equivalent to 0.62 ppm in the diet. The animal (and a control animal) was sacrificed 24 hours after the last dose. Samples of urine, feces, milk and blood were obtained daily. Tissue samples were obtained shortly after sacrifice of the animal. Milk samples were partitioned into four fractions: non-extractables, hexane solubles, ethyl acetate solubles, and aqueous solubles. Urine samples were analyzed by a 2-D paper process, the first dimension developed by electrophoresis, the second by ascending chromatography. No further information regarding the analytical methods is provided.

An overall low recovery of radioactivity of 73.7% was attributed to incomplete mixing of urine samples prior to sampling for radioassay. Most of the applied radioactivity was excreted in feces and urine with 0.4% found in milk and 0.2% found in tissues. Residues in feces and milk plateaued between days 2 and 4 (maximum residue in milk was 0.003 ppm). In milk samples on day 10, most of the residue was ethyl acetate (46%) or water (50%) soluble with only a small percentage of non-extractable (4.6%) and hexane extractable (0.2%) residues. TLE-TLC analysis of urine indicates that 80% of the urine residue was unchanged G-34048, 7% GS-17794 (deethylated hydroxymetabolite), 10% GS-17792 (deisopropylated hydroxymetabolite), and 3% G-35713 (ammelide, 2-amino-4,6-dihydroxy-s-triazine). Total radioactivity (hydroxyatrazine equivalents) in tissues were spleen (0.009 ppm), liver (0.007 ppm), kidney (0.004 ppm), heart (0.0008 ppm), muscle (round and tenderloin, 0.0006 ppm), brain (<0.0005 ppm), and fat (renal, subcutaneous, tail head, <0.0005 ppm).

GAAC-71021 (Part I) (MRID No. 412098-06)

Corn was treated preemergence with uniformly ring labeled ^{14}C atrazine at a rate equivalent to 3.0 lbs.a.i./A. Corn was also treated with non radioactive atrazine in the same manner. To produce corn silage, radioactive corn was chopped and placed in one gallon glass jars which were placed in a silo with non-radioactive corn so that all of the silage would be treated in

the same manner. Radioactive silage was mixed with Purina Goat Chow and molasses and fed to a lactating goat at a rate corresponding to 0.95 ppm in the diet for 5 days. Urine, feces, milk, and blood samples were collected daily. Tissue samples were collected approximately 20 hours after the final dose. Overall recovery of radioactivity was 88.4%, most of the radioactivity being excreted in urine and feces. Residues in milk plateaued by day 3 with a maximum residue of 0.004 ppm atrazine equivalents. Residues in tissues were liver (0.01 ppm), kidney (0.003 ppm), fat (0.0008 ppm), muscle (0.0008 ppm), and heart and brain (<0.0006 ppm).

TLC analysis of corn silage used for feed indicates that approximately 44% is composed of hydroxyatrazine (G-34048, 24-28%) or the mono-N-dealkylated hydroxymetabolites (GS-17794, 12-13%, GS-17792, 4-6%). Unknown aqueous soluble metabolites accounted for 27-34% of the residue, and 23-27% of the residue was non-extractable. The report states that a minimum of 87% of the residue was present as the triazine ring and "probably all of it was" (in feces). The compositions of the radiolabel in silage (feed) and goat urine (or urine plus feces combined) were compared to determine the extent to which the residue was modified by the goat. The results indicate that the residue is modified to some extent, but the hydroxymetabolites (G-34048, GS-17792, and GS-17794) still comprise a major portion of the residue (ca. 40-50%), and the remainder of the residue is composed primarily of unidentified water soluble metabolites.

GAAC-71021 (Part II) (MRID No. 412098-06)

Corn in a field plot was treated preemergence with uniformly ring labeled ^{14}C atrazine at a rate equivalent to 3.0 lbs.a.i./A. The total radioactive residue in harvested corn grain was 0.026 ppm (3.5 % chloroform soluble, 75% aqueous methanol soluble, and 22% non-extractable, discussed previously in the Second Round Review of the Registration Standard under Study No. GAAC-71022). The grain comprised 46% of the feed for a lactating goat (0.012 ppm in the goats diet) which was fed the radiolabelled grain for 5 days. Total recovery of the applied radioactivity was 76%. Most of the applied radioactivity was excreted in urine and feces with 1.4% recovered from milk, and 0.2% from tissues. Residues in milk plateaued by day 2 with a maximum residue of 0.00014 ppm. Residues in heart and liver were 0.0006 ppm, and residues in kidney, fat, muscle, and brain were all less than the limit of detection of 0.0006 ppm. Characterization of residues was not attempted.

GAAC-72088 (MRID No. 412098-06)

Milo sorghum was treated preemergence in the greenhouse with uniformly ring labeled ^{14}C atrazine at a rate of 2.5 lbs.a.i./A. Total radioactivity in fodder harvested at maturity was 8.9 ppm atrazine equivalents. The sorghum fodder was combined with goat

chow and molasses to be used as goat feed. A lactating goat was given this feed which she ate for the first two days of the study, refused on day three, and was fed in capsulated form on days 4-8 at a total average dosing rate equivalent to 1.47 ppm in the feed. Urine, feces, milk, blood, and CO_2 samples were collected daily. Tissue samples were collected at sacrifice (day 9). Plant, urine and feces samples were extracted with methanol:water, and the extract was further partitioned using a silica gel column and stepwise gradients of hexane / ethyl acetate / acetonitrile / methanol / water. Fractions from the silica gel column were analyzed using ion exchange chromatography to compare the elution patterns of sorghum fodder and animal excrement. TLC-TLE was carried out to quantify residues of hydroxymetabolites in these fractions.

Total recovery of radioactivity was 88.3% of which 69.3% was in feces, 15.0% in urine, 6.2% in the rumen, and 2.6% in expired CO_2. Because of the irregular feeding pattern, the day at which residues plateaued in milk and urine could not be accurately determined. The maximum residue found in milk was 0.003 ppm (day 8). Residues in tissues were liver (0.068 ppm), kidney (0.015 ppm), brain (0.005 ppm), heart (0.005 ppm), and muscle (0.002 ppm).

All of the residue in urine was extractable with 98% of the residue eluting in the acetonitrile:methanol eluate of the silica gel column. 45% of the residue in feces was extractable, and 68% of the residue in fodder. The neutral eluate from the ion exchange chromatography column was shown to contain G-34048 (1.3% feces, 4.7% fodder) and GS-17792/GS-17794 (3.4% urine + feces, 5.7% fodder, percentages of total applied radioactivity). Most of the extractable, neutral metabolites were not identified, and characterization of residues in other samples was not attempted. Comparison of the sephadex and ion exchange column results shows some common peaks among fodder, urine and feces samples, while some major peaks are not common to the samples.

ABR-89006 (MRID No. 412098-07)

Corn was treated preemergence with uniformly ring labeled ^{14}C atrazine at a rate of 3.0 lbs.a.i./A. Corn was harvested at the dent stage (60% of the crop) and at maturity (40% of the crop), the latter sample used in this study. The corn grain was field dried and fed to 4 hens at a level of approximately 0.047 ppm in the diet for 7 days. Eggs and excreta were collected daily, CO_2 and volatiles were collected for a single hen on days 2 and 6, and tissues and blood samples were collected at sacrifice.

Corn grain was extracted with 80:20 methanol:water, and the wet cake was then extracted with 50:50 methanol:water. The methanol:water filtrates were combined and then partitioned against ethyl acetate producing two final fractions (aqueous from methanol:water extract, and ethyl acetate). Excreta samples (hen 4, day 1) were treated similarly. Cation exchange chromatography

was performed on the aqueous fractions from both the corn grain and excreta.

Recovery of total radioactivity was 47.4%. This low recovery is attributed to the low levels present in the hens' feed. Most of the applied radioactivity was recovered from excreta (43.3%) with eggs and tissues accounting for 2.0% and 1.2% respectively. Residues in eggs did not plateau in the 6-day test period. Maximum levels in egg yolks and whites were 0.01 ppm and 0.008 ppm respectively. Maximum levels in edible tissues were liver (0.013 ppm) and kidney (0.009 ppm). Residues in lean meat, fat, and skin/attached fat are all reported as below the limit of detection, but no limit of detection for the method was provided.

Extraction characteristics and cation exchange chromatography histograms were compared for corn and day 1 excreta from a single hen. Extraction characteristics for excreta/corn (% recovered) were organosoluble (2.1%/4.4%), aqueous soluble (37.3%/40.5%), and non-extractable (22.5%/65.8%). A comparison of cation exchange chromatography histograms shows some common peaks, and some peaks present in corn which are not present in excreta. The submitter concludes that "analysis of excreta and grain extracts showed only minor changes in metabolic patterns by the chicken". Based on the limited amount of data available (histograms compare aqueous extracts only, no comments on the decreased percentage of non-extractable residues in excreta relative to corn, the differences in the histograms, and the various metabolites which would elute at similar retention volumes), we cannot conclude that the changes in the composition of the atrazine residue are minor resulting from metabolism of corn biosynthesized atrazine residues by chickens.

ABR-89054 (MRID No. 412098-08)

Corn was treated preemergence with uniformly ring labeled ^{14}C atrazine at a rate equivalent to 3.0 lbs.a.i./A. Corn grain was harvested at the dent stage and dried for use in this study. Two goats were fed the corn grain as 30% of their diets at levels of 0.30 ppm and 0.33 ppm (average = 0.32 ppm) in the total diet for six days. Urine, feces and milk samples were collected daily. Samples or CO_2 and volatile were collected on days 2, 4, 5 and 6. Blood samples were taken on days 2, 4, 6 and 7. Tissue samples were collected at sacrifice, 4 hours after the final dose. Sample storage times were not provided. Corn grain was extracted with methanol:water (90:10), the methanol was stripped, and the aqueous fraction was separately partitioned against hexane and ethyl acetate. The hexane and ethyl acetate fractions were analyzed by TLC (two solvent systems). Goat urine, feces and rumen samples were extracted using the biphasic extraction method described in Ciba-Geigy SOP 4.65. Aqueous soluble residues of corn grain and animal tissues were analyzed by cation exchange chromatography.

Overall recovery of applied radioactivity was 92.2%, most of which was recovered from the feces (average for 2 goats = 44.4%), urine (29.3%), and rumen (16.1%). Approximately 1.3% was recovered as CO_2 indicating that a small amount of triazine ring cleavage occurs. Residues in urine plateaued by day 5, while residues in milk and feces increased throughout the 6-day sampling period. The maximum residue found in milk was 0.003 ppm. Maximum residues found in liver and kidney were 0.036 ppm and 0.01 ppm respectively. Residues were below the limit of detection (=0.006 ppm) in brain, tenderloin, leg muscle, omental fat and perirenal fat.

Characterization of dent stage corn grain residues indicates that organic, aqueous, and non-extractable residues account for 6.5%, 60% and 27% of the total residue respectively. TLC analysis of the organic solubles, separated into hexane and ethyl acetate soluble fractions, indicate the presence of G-28273 (0.005 ppm), G-30033 (0.002 ppm), G-28279 (0.001 ppm), atrazine (trace), GS-17794 and GS-17792 (ppm values represent residues in ethyl acetate fraction only, hydroxymetabolites were not quantified).

Cation exchange chromatography histograms of the aqueous soluble corn residue suggest the presence of primarily GS-17794 and GS-17792. Histograms of goat urine, feces and rumen shows the presence of these same hydroxymetabolites plus a mixture of weakly basic metabolites which were not further characterized. These results indicate that the hydroxymetabolites and other biosynthesized metabolites of atrazine are further metabolized by the goat.

Attachment: Appendix
cc with attachment: M. Metzger (DEB), TOX II, atrazine S.F., J. Kariya (DRES/SACB), Circu(7), RF, atrazine SRR Reg Std. File, C. Furlow (PIB/FOD, H7506C, Rm.242)
RDI:F.Suhre:FS:4/10/90:RDS:4/11/90
H7509C:DEB:M.Metzger:MM:Rm803a:CM#2:4/10/90

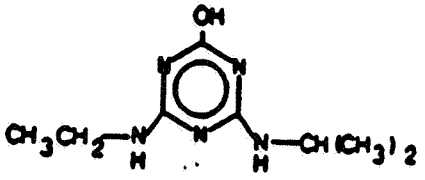
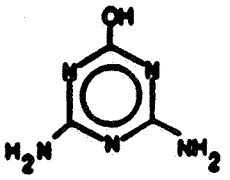
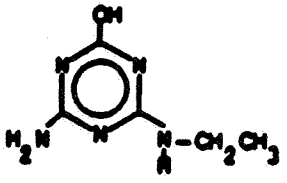
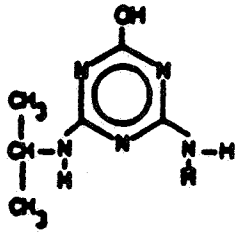
Appendix

Structures of Atrazine Metabolites

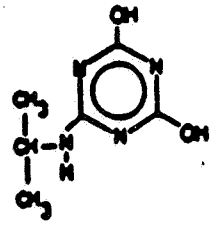
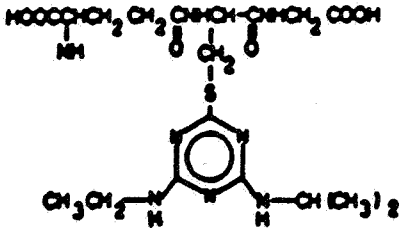
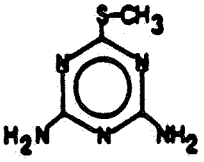
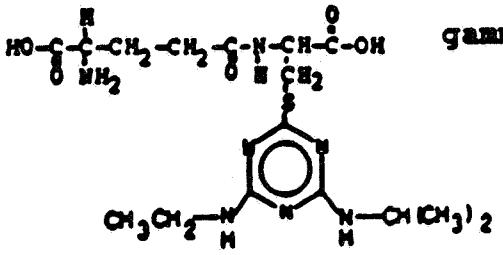
Atrazine and its metabolites in plants and animals.

Code	Chemical name Structure	Substrate	MRID Common name
I	2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine	Plants & Animals	00161854 Atrazine, G-30027
II	2-amino-4-chloro-6-isopropylamino-*s*-triazine	Plants & Animals	00161854 G-30033
III	2-amino-4-chloro-6-ethylamino-*s*-triazine	Plants & Animals	00161854 G-28279
IV	2,4-diamino-6-chloro-*s*-triazine	Plants & Animals	00161854 G-28273

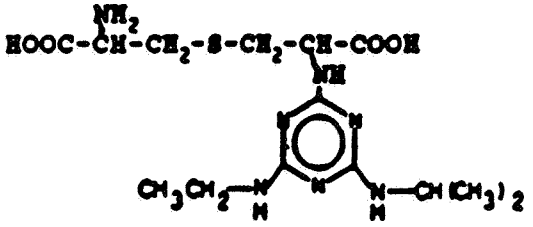
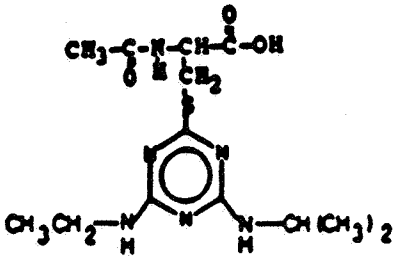
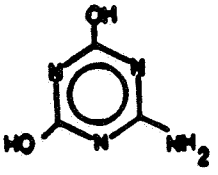
Atrazine and its metabolites (continued).

Code	Chemical name Structure	Substrate Common name	MRID
V	2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine 	Corn Sorghum **hydroxyatrazine, G-34048**	00161854 00161854
VI	2,4-diamino-6-hydroxy-*s*-triazine 	Goat milk Corn **Ammeline, GS-17791**	00161854 00161854
VII	2-amino-4-hydroxy-6-ethylamino-*s*-triazine 	Corn Sorghum **GS-17792**	00161854 00161854
VIII	2-amino-4-hydroxy-6-isopropylamino-*s*-triazine 	Corn Sorghum **GS-17794**	00161854 00161854

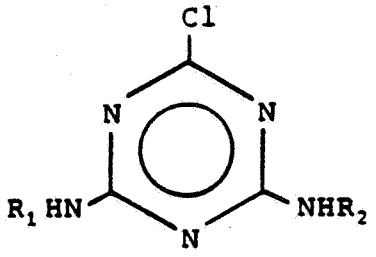
Atrazine and its metabolites (continued).

Code	Chemical name Structure	Substrate Common name	MRID
IX	2,4-(bis)hydroxy-6-isopropylamino-*s*-triazine 	Sorghum GS-11957	00161854
X	S-(4-ethylamino-6-isopropylamino-*s*-triazin-2-yl) glutathione 	Corn Sorghum glutathione conjugate	00161854 00161854
XI	2,4-diamino-6-(methylthio)-*s*-triazine 	Chicken egg,tissue GS-26831	40431357
XIV	gamma-glutamyl-*S*-(4-ethylamino-6-isopropylamino-*s*-triazin-2-yl) cysteine 	Cow liver gamma-glutamylcysteine conjugate	40437502

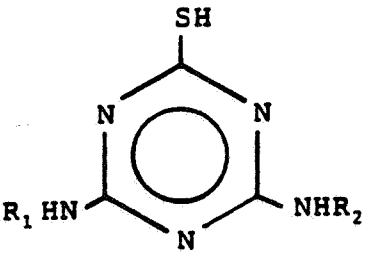
Atrazine and its metabolites (continued).

Code	Chemical name Structure	Substrate Common name	MRID
XV	2-[*N*-(bis)(2-amino-2-carboxyethyl)sulfide]-4-(ethylamino)-6-(isopropylamino)-*s*-triazine 	Sorghum lanthionine conjugate	00161854
XVI	2-(*N*-acetylalanyl-3-thio)-4-(ethylamino)-6-(isopropylamino)-*s*-triazine 	Sorghum mercapturic acid conjugate	00161854
	2-amino-4,6-dihydroxy-s-triazine 	412098-06 Ammelide, G-35713	

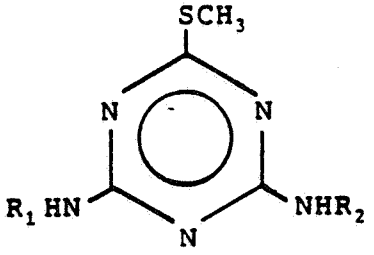
Additional Atrazine Metabolites Reputedly Found in Animals Tissues (MRID Nos. 412098-03, 412098-04)



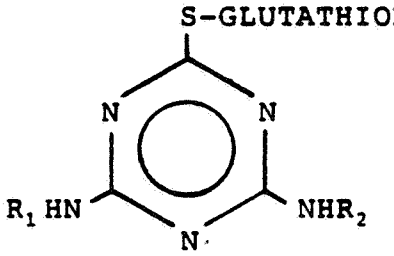
I. $R_1 = C_2H_5$, $R_2 = C_3H_7$
II. $R_1 = C_2H_5$, $R_2 = H$
III. $R_1 = H$, $R_2 = C_3H_7$
IV. $R_1 = H$, $R_2 = H$



IX. $R_1 = C_2H_5$, $R_2 = C_3H_7$
X. $R_1 = C_2H_5$, $R_2 = H$
XI. $R_1 = H$, $R_2 = C_3H_7$
XII. $R_1 = H$, $R_2 = H$



XIV. $R_1 = C_2H_5$, $R_2 = C_3H_7$
XV. $R_1 = C_2H_5$, $R_2 = H$
XVI. $R_1 = H$, $R_2 = C_3H_7$
XVII. $R_1 = H$, $R_2 = H$



XVIII. $R_1 = C_2H_5$, $R_2 = C_3H_7$
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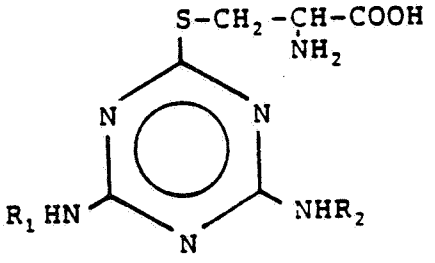

XXIII. $R_1 = C_2H_5$, $R_2 = C_3H_7$
XXIV. $R_1 = C_2H_5$, $R_2 = H$
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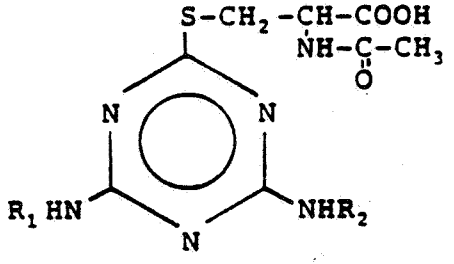
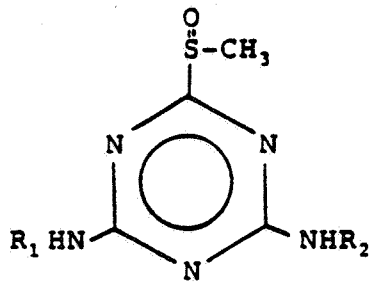
XXVI. $R_1 = H$, $R_2 = H$



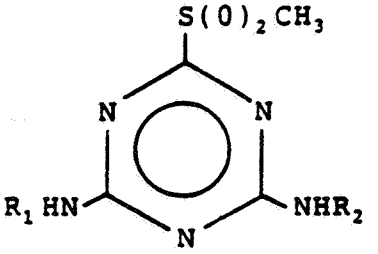
XXVII. $R_1 = C_2H_5$, $R_2 = C_3H_7$
XXVIII. $R_1 = C_2H_5$, $R_2 = H$
XXIX. $R_1 = H$, $R_2 = C_3H_7$
XXX. $R_1 = H$, $R_2 = H$

* We note that the numbering system used above applies only to MRID Nos. 412098-03, -04, and is inconconsistent with numbering used elsewhere.

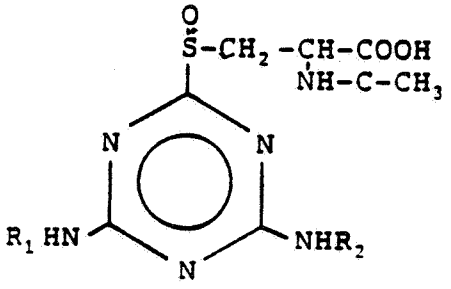
Additional Atrazine Metabolites Reputedly Found in Animals Tissues (MRID Nos. 412098-03, 412098-04)



XXXI. $R_1 = C_2H_5$, $R_2 = C_3H_7$
XXXII. $R_1 = C_2H_5$, $R_2 = H$
XXXIII. $R_1 = H$, $R_2 = C_3H_7$
XXXIV. $R_1 = H$, $R_2 = H$



XXXVII. $R_1 = C_2H_5$, $R_2 = H$
XXXVIII. $R_1 = H$, $R_2 = C_3H_7$
XXXIX. $R_1 = H$, $R_2 = H$



XLVII. $R_1 = C_2H_5$, $R_2 = H$
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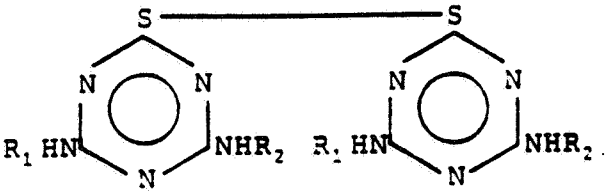
XLIX. $R_1 = H$, $R_2 = H$



XLII. $R_1 = C_2H_5$, $R_2 = C_3H_7$

* We note that the numbering system used above applies only to MRID Nos. 412098-03, -04, and is inconconsistent with numbering used elsewhere.