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

JUL 6 95

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Atrazine (080803), Reregistration Case No. 0062. Special Review. Ciba-Geigy Comments on the Triazine PD1; Additional Data on Metabolism in Sugarcane.
CBRS No. 15632, DPBarcode No. D215500, MRID 43598628.

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Special Review of triazine herbicides, including atrazine, has been initiated (59 FR 60412, 11/23/94, PD1). Ciba-Geigy Corporation has submitted comments in response, including additional data on the nature of the residue in sugarcane; the present submission represents Volume 29 of registrant's comments. Assignment instructions are to review the present submission in response to the PD1 and provide evaluation for PD2/3. The conclusions below pertain to the present submission, and its relation to the Agency position in the PD1.

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)). Designations for the metabolites in the tolerance expression are G-28279, G-30033, and G-28273, respectively; structures are indicated in Figure 1. 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 Related to This Submission

1. The previous submission demonstrated that atrazine undergoes extensive metabolism in sugarcane. Registrant identified chloro, hydroxy, amino, and conjugated metabolites, each containing an intact triazine ring (see Figure 1 here for details). Using Extraction Method I (initial extraction in methanol:water), residues identified represented 26.9% TRR in final harvest leaves, and 52.5% TRR in final harvest cane. Atrazine and other chloro compounds represented 5% TRR in final harvest cane, and less than 2% in final harvest leaves. (CBRS 12889, 6/29/95, J. Abbotts).

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

3. With the previous submission and the present submission, registrant has identified a total of 21 metabolites, each containing an intact triazine ring, together accounting for 45% of TRR in sugarcane leaves (see Figures 1 through 4 and Table 1 for details). One metabolite represented 11% TRR; all others individually represented less than 10% TRR.

Conclusion with Regard to the PD1

The Agency position on plant metabolism can be summarized in the following manner: Atrazine metabolism in plants is extensive, no single metabolite represents a large portion of the total triazine residue, analytical methods to measure total triazine ring residues are not available, and therefore, total radioactive residues from radiolabel field studies are the most appropriate data to use for risk assessment. The previous and present submissions on metabolism in sugarcane indicate that atrazine metabolism is extensive, no single metabolite represents a large portion of the total residue, and each metabolite identified contains an intact triazine ring. This information does not contradict the Agency position, and in fact reinforces it.

CBRS 15632, PD1 Comment, Atrazine in Sugarcane, p. 3 of 12

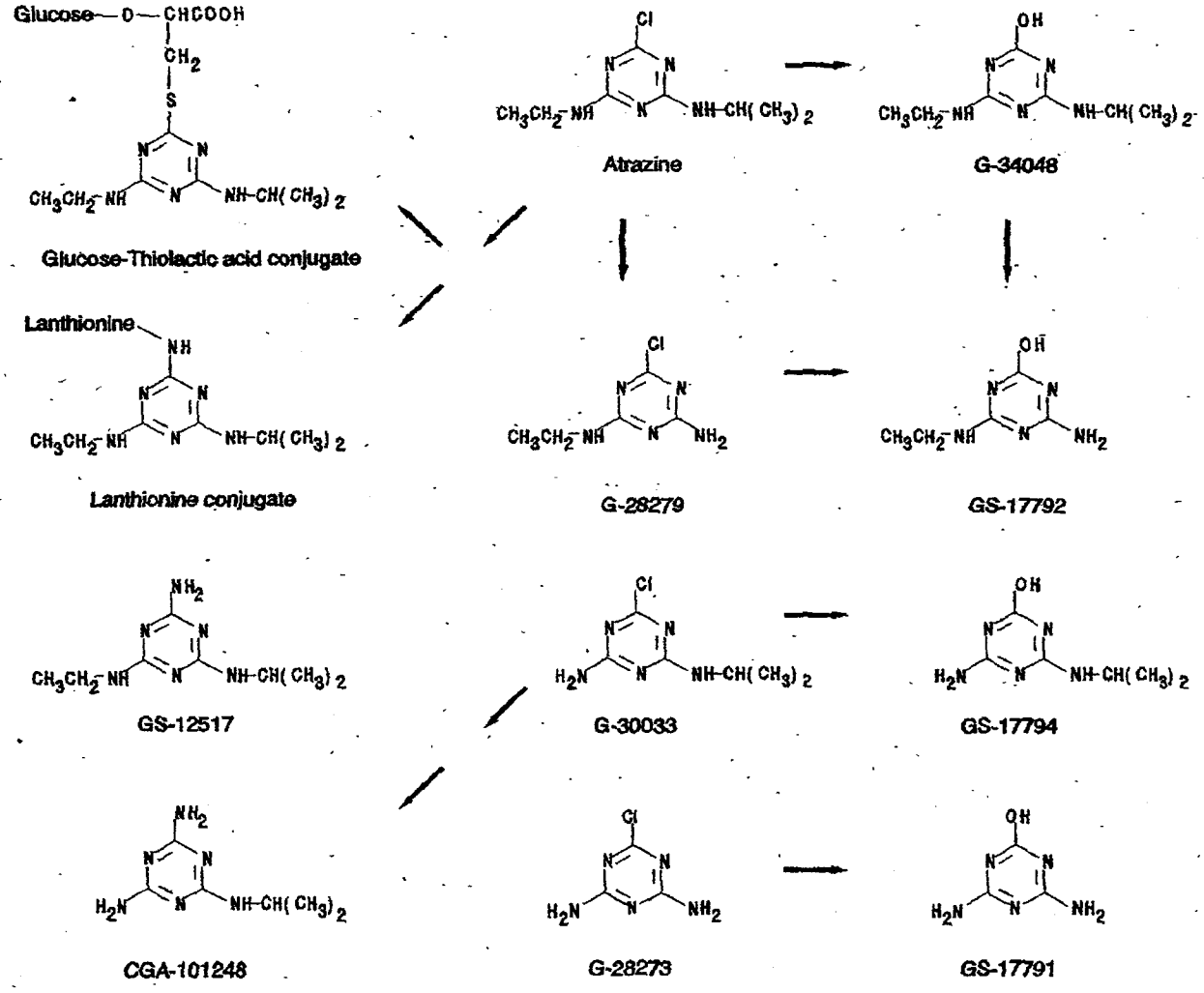


Figure 1. Atrazine metabolites previously identified in sugarcane.

DETAILED CONSIDERATIONS

PD1 Position on Plant Metabolism

The initiation of special review on the triazines describes the Agency's position pertaining to triazine metabolism and residues of concern. The full FR notice contains more detail, but the following excerpts outline the Agency position (59 FR 60412, 11/23/94):

"In estimating triazine dietary risks, the Agency assumes that the total toxic residue of concern is the parent triazine compound plus all metabolites with a triazine ring, including among others, all chloro and hydroxy metabolites."

"In plants, atrazine and simazine are metabolized to numerous metabolites, no one of which has yet been shown to comprise a large portion of the total terminal residue.... Most metabolites have been shown to contain the intact triazine ring." (59 FR 60418)

"Based on its assessment of the structure-activity relationship and potential carcinogenicity of all registered triazine compounds, EPA believes metabolites which have been dechlorinated may be less potent carcinogens than the parent compounds.... However, in the absence of completed laboratory studies of the hydroxy metabolites, the Agency has relied on its equivalency policy and has made the assumption that all metabolites containing the triazine ring are equipotent as carcinogens as the parent compound when conducting its risk assessment." (59 FR 60418-60419)

"Since the registrants have been unable to develop analytical methodology which measures total triazine ring residues in non-radiolabel field trials, radiolabel field studies currently provide the best data to use for risk assessment. New radiolabel field studies for major dietary risk contributors for both atrazine and simazine have been submitted to the Agency and are currently under review." (59 FR 60419)

The Agency position can be summarized in the following manner: atrazine metabolism in plants is extensive, no single metabolite represents a large portion of the total triazine residue, analytical methods to measure total triazine ring residues are not available, and therefore, total radioactive residues from radiolabel field studies are the most appropriate data to use for risk assessment. The present submission will be reviewed with regard to this Agency position.

Background

In response to an Agency DCI of 10/90 requiring radiolabel field studies, registrant submitted field metabolism studies in sugarcane. This previous submission has recently been reviewed (CBRS 12889, 6/29/95, J. Abbotts). Figure 1 indicates the residues identified in sugarcane by the previous submission. The left side of the figure indicates residues believed to be formed following conjugation with glutathione, and the multiple arrows indicate that the residues identified were formed from rearrangement or additional metabolism of the glutathione conjugate. The individual residues in Figure 1 were identified after use of Extraction Method I, where the initial extraction was in methanol:water. Conclusions from review of the previous submission on sugarcane that are relevant to review of the present submission are repeated here:

Conclusion 1: The previous submission demonstrated that atrazine undergoes extensive metabolism in sugarcane. Registrant identified chloro, hydroxy, amino, and conjugated metabolites, each containing an intact triazine ring (see Figure 1 here for details). Using Extraction Method I (initial extraction in methanol:water), residues identified represented 26.9% TRR in final harvest leaves, and 52.5% TRR in final harvest cane. Atrazine and other chloro compounds represented 5% TRR in final harvest cane, and less than 2% in final harvest leaves. (CBRS 12889, 6/29/95, J. Abbotts).

Conclusion 2: In the previous submission on sugarcane, with Extraction Method II, an acid autoclave procedure, approximately two-thirds of the TRR in final harvest leaves and cane was converted to cyanuric acid and the atrazine hydroxy metabolites G-34048, GS-17794, and GS-17792 (Ibid.). This observation indicates that the position of the HED Metabolism Committee, that TRR should represent total residues containing the triazine ring, is a reasonable assumption.

As part of its response (Volume 29 of 54) to the FR notice initiating special review, registrant submitted the following document:

^{14}C-Atrazine: Nature of the Residue in Sugarcane, Supplement No. 1 to the Final Report, Study Completed on 10/18/93, Ciba-Geigy Corporation, Greensboro, NC (MRID 43598628).

The performing laboratory for the original study, as well as for this supplement, was Hazelton Wisconsin, Inc., Madison, WI. Supplement No. 1, the present submission, describes additional laboratory work pertaining to identification of residues in sugarcane leaves collected prior to final harvest.

Additional Laboratory Analysis

In the original metabolism study, four applications were made to sugarcane, the last 137 days before harvest. Pre-fourth application leaves were collected, and residues were identified in these samples as well as in mature cane and leaves. Total radioactive residues in pre-fourth applications leaves were 69 ppm, higher than in mature leaves (24 ppm) and mature cane (2 ppm). The registrant reopened the sugarcane metabolism study to determine if additional metabolites could be identified in pre-fourth application leaves.

In the original metabolism study, with Extraction Method I, sugarcane samples were extracted in aqueous methanol. The total extract from this step was then partitioned with chloroform, and the aqueous extract was examined by Aminex A-4 cation exchange chromatography. These same techniques were used in the present submission, and Aminex fractions designated Peak 1 and Peak 8 from 21 column runs were collected separately and combined for each of these two peaks.

The combined Aminex Peak 1 sample was further fractionated by octadecyl chromatography, using a C18 column, eluted with step gradients in 1% acetic acid in water (Solvent A) and 1% acetic acid in acetonitrile (Solvent B). This produced six separate C18 peaks, some of which were not well resolved. Additional analysis of the C18 peaks by HPLC produced additional peaks that were further purified and in some cases analyzed by liquid chromatography/mass spectrometry, using a VG Quattro mass spectrometer, operated in either electrospray positive ionization mode or electrospray negative ionization mode.

Aminex Peak 8 contained ammonium formate, which was removed by additional Aminex A-4 chromatography. Analysis of Peak 8 by HPLC, using a YMC-Pack AQ-303 ODS column, eluted with gradients in Solvents A and B described above, produced two major peaks. These were purified by further HPLC chromatography and analyzed by liquid chromatography/mass spectrometry.

Residues from C18 peaks and Aminex Peak 8 were analyzed by acid hydrolysis. When the product of hydrolysis was G-34048 (hydroxyatrazine), this indicated that the original residue was a conjugate with the Cl atom in atrazine displaced. When products of hydrolysis were G-34048 and GS-12517, an amine linkage in the original conjugate was indicated. When the product of acid hydrolysis was GS-17794, a conjugate through G-30033 was indicated.

Based on mass spectra, and consistent with the results of acid hydrolysis, the Registrant proposed the following residues from the C18 chromatography of Aminex Peak 1 (see Figures 2 through 4 for structures):

cysteic acid conjugate of atrazine;
S-acetate-N-cysteinyl sulfoxide conjugate of atrazine;
S-lactate-N-cysteinyl sulfoxide conjugate of atrazine;
glucose-thiolactate conjugate of atrazine (which was also identified in the previous submission);
N-malonyl-cysteinyl conjugate of atrazine;
S-lactate-N-cysteinyl conjugate of atrazine;
O-malonyl-S-lactate-N-cysteinyl conjugate of G-30033; and
glucose-thiolactate conjugate of G-30033.

Based on mass spectra, and consistent with the results of acid hydrolysis, the Registrant proposed that the two major residues from Aminex Peak 8 were:
an N-homocysteine glucose ester conjugate of G-30033, and a glucoside conjugate of GS-17794 (see Figure 4 for structures). With the latter residue, data were insufficient to determine whether the metabolite was an O- or N- glucoside.

Figures 2 through 4 show structures of the residues identified in the present submission. With the exception of the GS-17794 glucoside, the Registrant proposed that these additional residues were formed through conjugation with glutathione. Under the proposed pathway, initial conjugation is through the SH group of glutathione. Internal rearrangement then produces an amine conjugate, and the amine bond remains while additional metabolism proceeds on the conjugated molecule. Figures 2 and 3 indicate proposed pathways for atrazine conjugates; these Figures show the full structures for the glucose-thiolactate and lanthionine conjugates, which are shown in abbreviated form in Figure 1. Figure 4 indicates the proposed pathway for conjugation with G-30033. The proposed metabolic pathways proceed through intermediates which were not isolated, and which are not shown here.

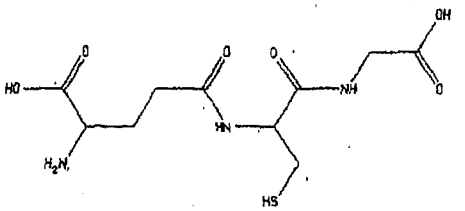
With this additional work, a total of 21 metabolites have been identified in sugarcane leaves. Table 1 summarizes the assignment of residues in leaves, including residues identified in the previous submission (CBRS 12889, 6/29/95, J. Abbotts), and in the present submission. Based on the assignment of residues, registrant concluded that a major pathway for atrazine metabolism in sugarcane involves glutathione conjugation.

Table 1. Assignment of residues in pre-fourth application sugarcane leaves.

Residue	% TRR
Data from the previous submission:	
Atrazine	0.2
G-30033	0.5
G-28279	0.2
G-28273	8.2
G-34048	0.7
GS-17794	8.5
GS-17792	0.9
GS-17791	1.8
CGA-101248	2.2
GS-12517	1.0
Lanthionine conjugate of atrazine	1.4
Glucose-thiolactate conjugate of atrazine	11.1
Data from the present submission:	
Cysteic acid conjugate of atrazine	0.6
S-Acetate-N-cysteinyl sulfoxide conjugate of atrazine	1.3
S-Lactate-N-cysteinyl sulfoxide conjugate of atrazine	0.9
N-Malonyl-cysteinyl conjugate of atrazine	0.1
S-Lactate-N-cysteinyl conjugate of atrazine	0.3
O-Malonyl-S-lactate-N-cysteinyl conjugate of G-30033	0.5
Glucose-thiolactate conjugate of G-30033	3.6
N-Homocysteine-glucose ester conjugate of G-30033	0.2
Glucoside conjugate of GS-17794	0.9
Total	45.1

Table notes: TRR was 69 ppm. Assignment of the first group of residues was from MRID 43016503, reviewed in CBRS 12889, 6/29/95. Structures are shown in Figures 1 through 4.

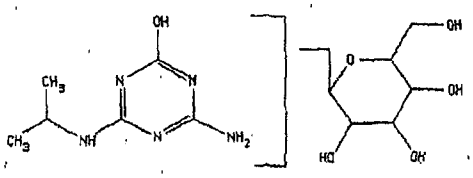
Atrazine



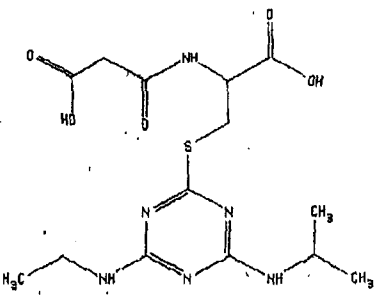
Glutathione

GS-17794

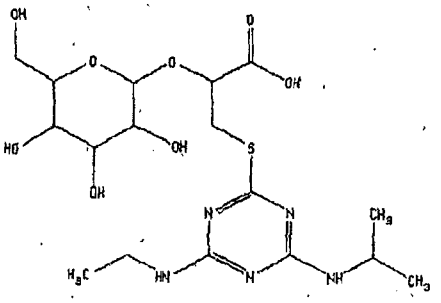
[Conjugation, additional metabolism]



O- or N-Glucoside of GS-17794

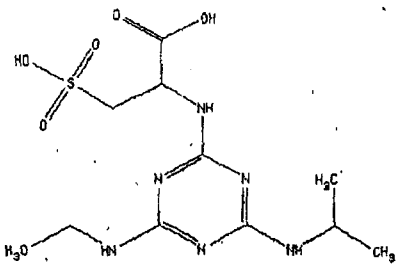


N-Malonyl-cysteinyl conjugate

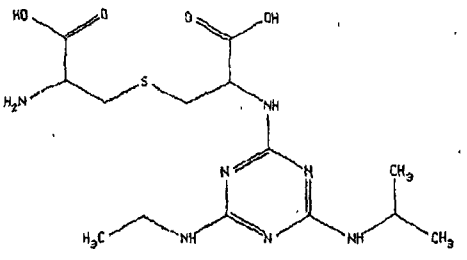


Glucose-thiolactate conjugate

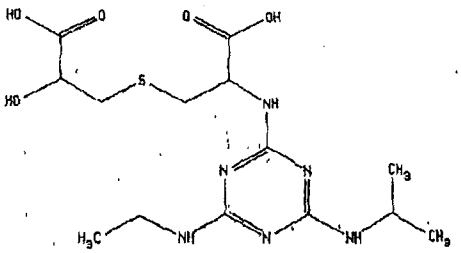
Figure 2. Metabolism in sugarcane leaves. Glucose conjugates and S-conjugates of atrazine from glutathione.



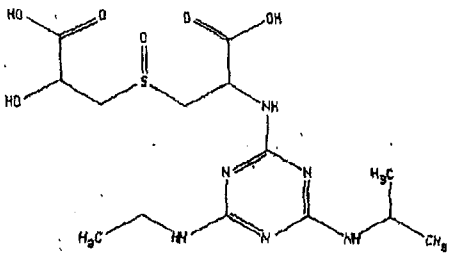
Cysteic acid conjugate



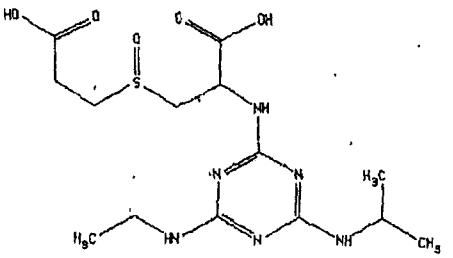
Lanthionine conjugate



S-Lactate-N-cysteinyl conjugate



S-Lactate-N-cysteinyl sulfoxide conjugate



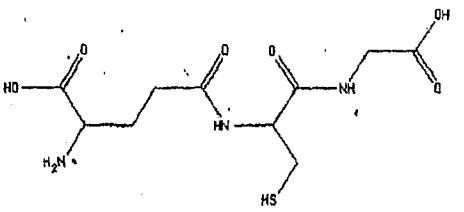
S-Acetate-N-cysteinyl sulfoxide conjugate

↓

GS-12517
(Unconjugated; see Figure 1)

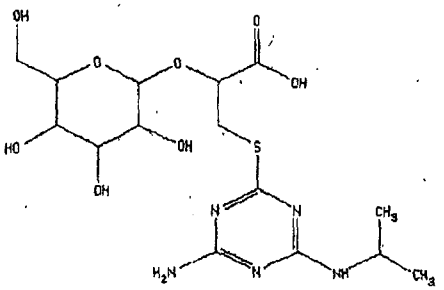
Figure 3. Metabolism in sugarcane leaves. N-conjugates of atrazine from glutathione.

G-30033 +

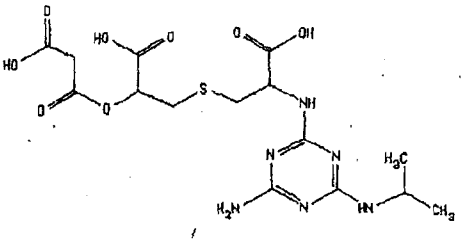


Glutathione

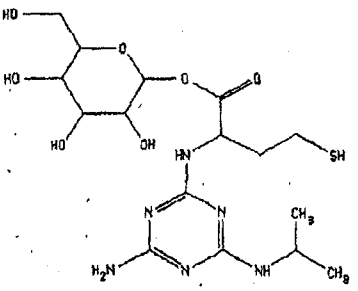
[Conjugation, rearrangement, and/or additional metabolism]



Glucose-thiolactate conjugate of G-30033



O-Malonyl-S-lactate-N-cysteinyl conjugate of G-30033



N-Homocysteine-glucose ester conjugate of G-30033

CGA-101248
(unconjugated; see Figure 1)

Figure 4. Metabolites of G-30033 in sugarcane leaves.

CBRS Comments, Additional Laboratory Work

Conclusion 3: With the previous submission and the present submission, registrant has identified a total of 21 metabolites, each containing an intact triazine ring, together accounting for 45% of TRR in sugarcane leaves (see Figures 1 through 4 and Table 1 for details). One metabolite represented 11% TRR; all others individually represented less than 10% TRR.

The Agency's position in the PD1 was described and summarized above (see section on PD1 Position on Plant Metabolism). The combined submissions on sugarcane do not contradict the Agency's position, and in fact reinforce it, leading to the following overall conclusion:

Conclusion with Regard to the PD1: The Agency position on plant metabolism can be summarized in the following manner: Atrazine metabolism in plants is extensive, no single metabolite represents a large portion of the total triazine residue, analytical methods to measure total triazine ring residues are not available, and therefore, total radioactive residues from radiolabel field studies are the most appropriate data to use for risk assessment. The previous and present submissions on metabolism in sugarcane indicate that atrazine metabolism is extensive, no single metabolite represents a large portion of the total residue, and each metabolite identified contains an intact triazine ring. This information does not contradict the Agency position, and in fact reinforces it.

cc:Circ, Abbotts, RF, Atrazine List A File, SF
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7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:7/5/95
JA12\atrazine.10