Triazine

7/9/92

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

JUL 7 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

7/9/92 12:00 Rm 817

SUBJECT: Metabolism of the Triazine Herbicides Atrazine, Simazine, and Cyanazine. Issues to be presented to the Metabolism Committee on 7/8/92.

FROM: Michael S. Metzger, Chemist
Chemistry Branch 2 - Reregistration Support
Health Effects Division (H7509C)

THRU: Edward Zager, Chief
Chemistry Branch 2 - Reregistration Support
Health Effects Division (H7509C)

TO: The HED Metabolism Committee
Health Effects Division (H7509C)

Background

SPECIAL REVIEW: As a result of a dietary risk assessment completed for atrazine in 1988 (and more recent revisions), a Grassley-Allen letter was sent to atrazine registrants notifying them that the Agency's was considering placing atrazine into Special Review. The most recent decision by EPA upper management indicated that the three chlorotriazine herbicides atrazine, simazine, and cyanazine should be considered together because:

- they have a common toxicological end-point;
- they have common metabolites; and
- restriction of use of one of these herbicides would likely lead to increased use of another to some extent.

The Special Review process is currently inactive in part because HED's position that all components with an s-triazine ring are of concern has raised difficult issues which both the registrants and the Agency are trying to resolve.

REREGISTRATION: Metabolism studies relating to triazine herbicides submitted to the Chemistry Branches in response to Reregistration DCIs present difficult issues. Best approaches to resolution of these issues are not apparent, and issuance of these reviews is delayed.

<u>Summary:</u>

Cyanazine is metabolized in plants to 9 specific metabolites, in both free and conjugated forms, representing a large portion (>90%) of the total radioactive residue (TRR). CBRS has concluded that, although the total triazine ring is of toxicological concern, regulation of these 9 metabolites is sufficient because such a large portion of the TRR and triazine ring is accounted for.

The metabolism of atrazine and simazine in plants is somewhat different from cyanazine in that:

- the metabolism studies are older, and metabolites are not adequately identified in many cases; and
- there appears to be more extensive metabolism in plants, i.e., there are a greater number of metabolites found, each at very low levels.

The major metabolites in plants are those resulting from:

√ (1) 2-hydroxylation (displacement of chlorine);

√ (2) loss of alkyl groups from the amine side-chains (except the cyano-containing side-chain of cyanazine which is modified but not lost);

(3) modification of the alkyl groups on the amine side chains (primarily on the cyano-containing side-chain of cyanazine);

(4) conjugation with glutathione followed by modification of the glutathione portion of the conjugate;

and possibly

(5) conjugation with other plant components including amino acids and sugars; and

(6) oxidation at the amine forming di-ring hydroxylated metabolites.

The Agency has issued extensive DCIs requiring **GEOGRAPHICALLY REPRESENTATIVE RADIOLABEL FIELD STUDIES FOR MOST REGISTERED COMMODITIES FOR ATRAZINE AND SIMAZINE.** The registrant has agreed to do most of these studies, and has made significant progress in these studies for atrazine.

Analytical methodology is not currently available (or likely to become available) which measures all metabolites of atrazine or simazine which contain an s-triazine ring. The registrant stated that they have tried to develop marker methodology for over 25 years, but have been unsuccessful because background triazine levels are too high. One goal of the required radiolabeled field studies is to determine if some type of marker methodology can be used to regulate these pesticides.

The dietary exposure assessments for atrazine and simazine done by CBRS utilizes the total radioactivity from metabolism studies for corn, sorghum, and animal products to determine dietary exposure from these commodities assuming most of the radioactivity reflects triazine moieties (highly probable). In most cases (notably excluding milk), the percentage of the total triazine residue accounted for by chlorotriazines is small for these commodities (ca. 3-15% organo-soluble residue depending on timing of application). [We note that <u>detectable</u> chlorotriazine residues are found in <u>cold</u> field trials for sugarcane requiring that this commodity possibly be examined/regulated separately.]

A Structure Activity Relationship analysis was prepared by the Office of Toxic Substances and reviewed by HED (Henry Spencer, Ph.D., 1/7/91). The SAR discusses the relative carcinogenic potencies of different s-triazines.

Atrazine is the #2 pesticide in the U.S. in terms of total use. 80-90% of atrazine is used on corn, 12% on sorghum, 1% on sugarcane, and the rest on wheat and other crops. Any cancellation of atrazine use on major crops would impact the registrant as well as farmers growing these crops.

<u>Questions to the Committee:</u>

(1) Considering the percentage of chlorotriazine residues found in corn and sorghum (<5-15%) as well as the results of the SAR, is the Committee sufficiently concerned about the dietary risk from atrazine and simazine to go forward with the Triazine Special Review utilizing the total radioactive residue for risk assessment? Or is the available risk assessment sufficiently uncertain (i.e. the risk may be less than that estimated using the total radioactive residue data with the q_1^*) such that further action regarding these pesticides should await submission of the required radiolabel field studies or other information?

(2) Will the results of the field radiolabel studies be of any use to TOX in determining dietary risk considering that we already have an estimate of the total triazine residue from previously submitted metabolism studies? Since TOX is not requiring further studies, should we proceed with review of these chemicals based on risk assessment using the total triazine residue?

(3) Will toxic effects other than carcinogenicity effect which metabolites of atrazine and simazine need to be regulated.

DETAILED CONSIDERATIONS

Below are summaries of atrazine and cyanazine plant metabolism studies. Little data is available for simazine, but its metabolism is likely to be similar to atrazine because of the similarities in structure.

ATRAZINE

Atrazine plant metabolism data were submitted only for corn and sorghum. Since the total toxic residue for atrazine is currently defined to include all metabolites with a triazine ring, unequivocal identification of all triazine ring-containing metabolites was determined to be unnecessary - analytical method development would require derivitization to a common moiety prior to analysis because of the large number of metabolites involved. Unambiguous structural determination was made for the 2-chloro and 2-hydroxy metabolites as well as for the glutathion conjugate. Identification of other metabolites was less rigorous, although it was determined that generally >95% of the total residue contained metabolites with the triazine ring. Below we present the available data for corn; metabolism studies for sorghum showed generally similar results although only small percentages of the radioactivity was identified in the sorghum metabolism studies.

Table 1 below summarizes atrazine metabolites found in corn. Metabolite structures are shown in Figure 1.

Table 1: Atrazine Metabolites in Corn: 0.5X Treatment Rate, Pre-emergence

<table>
<tr><td></td><td colspan="5">Percent (ppm)</td><td></td></tr>
<tr><td>Time (weeks)--></td><td>4</td><td>11</td><td colspan="3">15</td><td></td></tr>
<tr><td>Plant Part ---></td><td>Whole Plant</td><td>Whole Plant</td><td>Stalks</td><td>Grain</td><td>Cobs</td><td></td></tr>
<tr><td>Total PPM ---></td><td>5.48</td><td>2.31</td><td>5.29</td><td>0.30</td><td>0.45</td><td></td></tr>
<tr><td>METABOLITE</td><td></td><td></td><td></td><td></td><td></td><td>ID[1]</td></tr>
<tr><td>GS-17794</td><td rowspan="2">13.1 (0.072)</td><td rowspan="2">48.4 (1.12)</td><td>32.7 (1.73)</td><td>9.1 (0.03)</td><td>21.3 (0.10)</td><td>S</td></tr>
<tr><td>GS-17792</td><td>6.4 (0.34)</td><td>2.5 (0.01)</td><td>7.5 (0.03)</td><td>S</td></tr>
<tr><td>G-34048</td><td>22.6 (1.24)</td><td>5.4 (0.12)</td><td>1.9 (0.10)</td><td>0.9 (0.01)</td><td>1.7 (0.01)</td><td>S</td></tr>
<tr><td>Cysteine conjugate</td><td>-</td><td>3.5 (0.08)</td><td>3.4 (0.18)</td><td>4.2 (0.01)</td><td>6.5 (0.03)</td><td>W</td></tr>
<tr><td>GS-10813</td><td>2.9 (0.16)</td><td>4.2 (0.10)</td><td>1.1 (0.06)</td><td>10.2 (0.03)</td><td>19.2 (0.09)</td><td>M</td></tr>
<tr><td>GS-11957</td><td>-</td><td>-</td><td>5.6 (0.30)</td><td>3.4 (0.01)</td><td>-</td><td>M</td></tr>
</table>

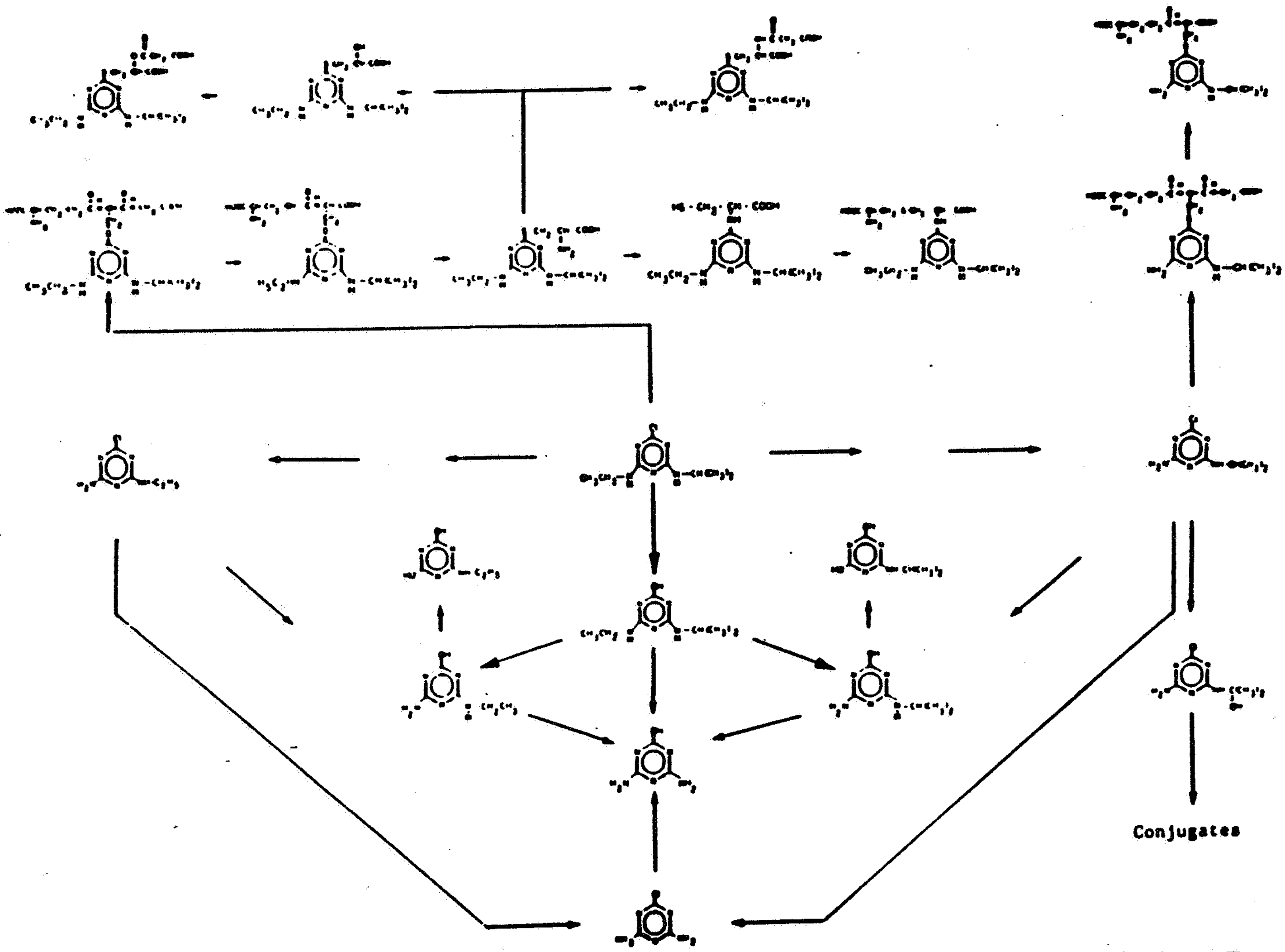
	Percent (ppm)					
Time (weeks)-->	4	11	15			
Plant Part --->	Whole Plant	Whole Plant	Stalks	Grain	Cobs	
Total PPM --->	5.48	2.31	5.29	0.30	0.45	
METABOLITE						ID[1]
Sugar conjugate of a hydroxy metabolite (2 components)	7.4 (0.41)	12.2 (0.03)	5.2 (0.28)	10.6 (0.03)	7.1 (0.03)	W
Glutathion conjugate	12.3 (0.67)	-	-	-	-	S
CGA-62373	-	1.1 (0.03)	-	-	-	M
Organo-soluble residue (includes chloro-metabolites)	2.0 (0.11)	5.6 (0.13)	0.5 (0.03)	6.8 (0.02)	0	-
TOTAL	60.3 (3.30)	80.4 (1.86)	56.8 (3.00)	51.9 (0.16)	63.3 (0.28)	
Other Unidentified metabolites						
Unknown	5.4 (0.30)	-	-	-	-	
Unknown	0.7 (0.04)	3.3 (0.08)	1.9 (0.10)	2.3 (0.01)	0.9 (<0.01)	
Unknown	3.1 (0.17)	3.1 (0.07)	1.3 (0.07)	2.1 (0.01)	-	
Unknown	1.3 (0.07)	-	-	-	-	
Unknown	-	-	5.2 (0.28)	-	-	

The registrant theorizes that the glutathion conjugate and any potential breakdown products of the glutathion conjugate containing the sulfur atom are further metabolized to the coresponding sulfoxides and sulfones. No other single metabolite other than those listed above accounts for >10% or >0.01 ppm in this metabolism study.

To summarize:

- The presence of hydroxy-metabolites results from: (a) uptake of hydroxyatrazine from the soil (hydroxyatrazine is a major soil metabolite, (b) non-enzymatic hydroxylation of atrazine in the roots, and (c) possibly from hydrolysis of the glutathion conjugate;
- N-dealkylation occurs in the shoots and leaves; and
- glutathion conjugation occurs in the shoots and leaves followed by modificaton of the glutathion side-chain to form mono- and di-peptides and related compounds, and oxidation of the sulfur to form sulfoxide and sulfone derivatives.

The proposed metabolic pathway in corn is summarized in Figure 2.



PROPOSED METABOLIC PATHWAY FOR ATRAZINE IN CORN. UPDATED VERSION OF ORIGINAL PATHWAY AS PROPOSED BY SHIMABUKURO

CYANAZINE

Cyanazine metabolites found in corn and wheat metabolism studies are summarized in Table 2. Three general processes are involved: (1) N-deethylation, (2) 2-hydroxylation, and (3) modification of the cyano group to form the amide and acid. The proposed metabolic pathway for cyanazine in plants is shown in Figure 2.

Table 2: Cyanazine Metabolites in Corn and Wheat

Residue	% Total Radioactive Residue (ppm)					
	Corn forage, pre-Emerg. at 0.28X	F. corn (0.37X), S. corn (0.2-0.74X), pre-Emerg, GH			Corn, Post-Emerg (0.2X), GH, fodder	Wheat, Pre-Emerg. (0.08-0.32X), GH, Straw + Leaves
		Ears	Stems	Leaves		
Cyanazine	<0.01 (<0.01)			2.1- 6.4 (<0.08)	0.5 (0.03)	0.3- 1.9
DW 4002	-			-	-	-
DW 3565	-			<0.5 (<0.01)	0.35 (0.02)	0.3- 1.9
DW 4006	25.1 (0.64)		•	5.6-23.4 (<0.33)	16.7 (0.95)	0.6- 2.8
DW 4510	-			-	-	14.2-26.1
DW 4394	43.8 (1.12)		•	18.3-22.6 (<0.39)	25.3 (1.44)	21.7-33.1
DW 3561	7.8 (0.20)			6.4-11.3 (<0.16)	16.7 (0.95)	4.4- 9.4
DW 3486	-			-	-	5.6-10
DW 4385	16.5 (0.42)		•	7.8-12.6 (<0.26)	13.0 (0.74)	10-18
Conjugated DW4385/DW4394	-			~34	-	-
Unidentified	-			<19	27.4 (1.56)	-
Unextractable	6.7 (0.17)			-	-	-
Total	100 (2.55)	(<0.02)	81-100	100 (0.31-2.07)	100 (5.69)	

In contrast to atrazine, a large percentage of the cyanazine residue in corn and wheat can be accounted for by the parent and the major chloro and hydroxy metabolites.

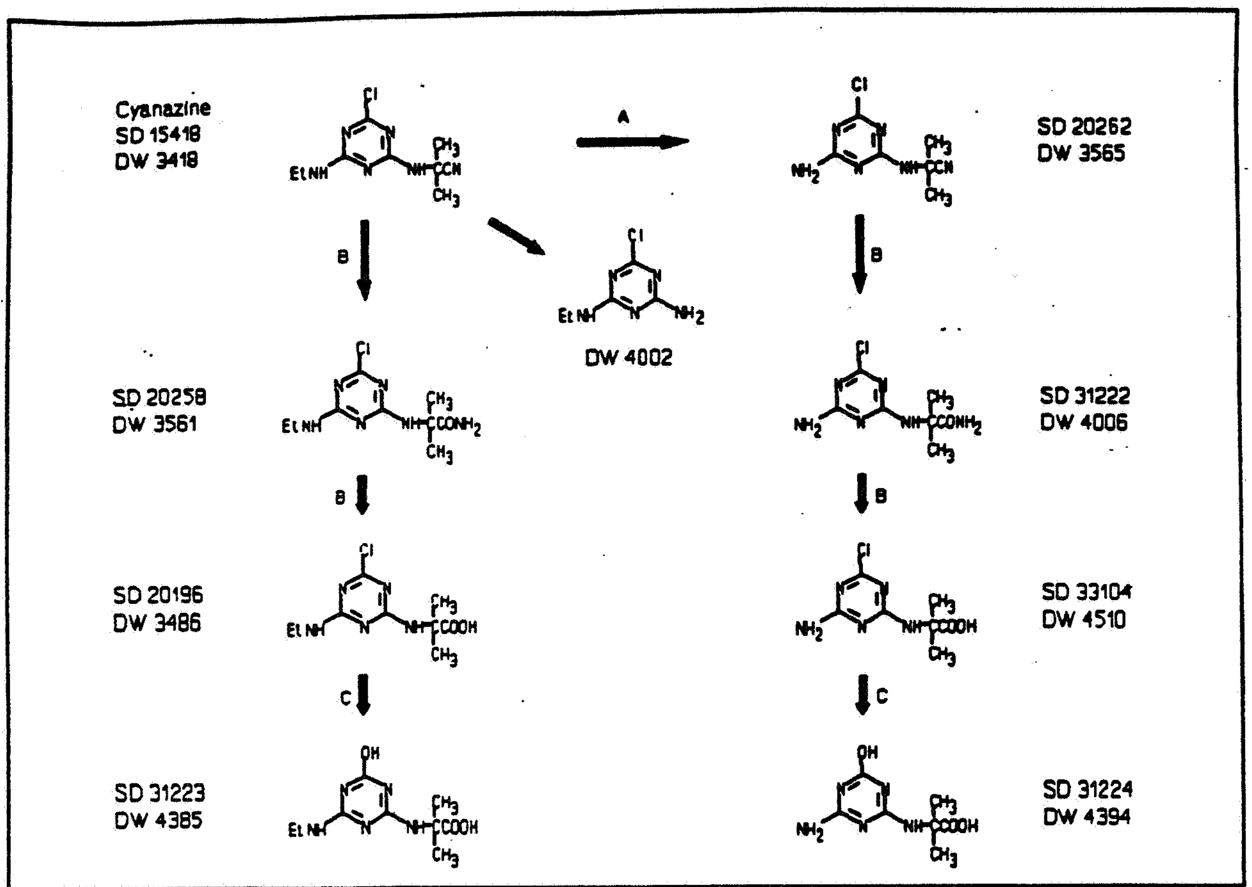
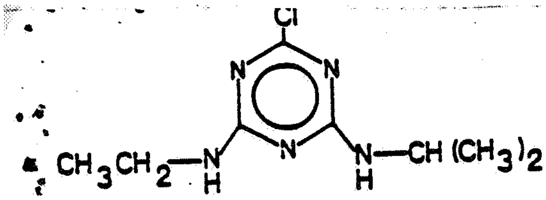
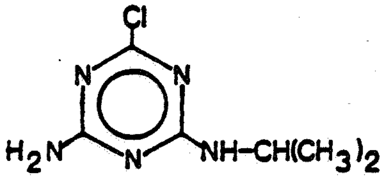


Figure 1. Plant Metabolism of Cyanazine

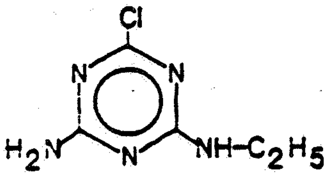


Atrazine
G-30027



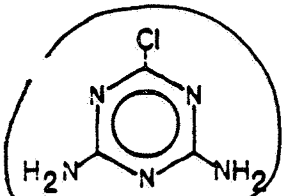
G-30033

2-Amino-4-chloro-6-isopropylamino-s-triazine



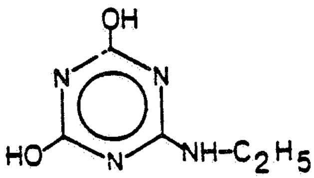
G-28279

2-Amino-4-chloro-6-ethylamino-s-triazine



G-28273

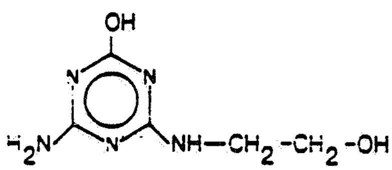
2,4-Diamino-6-chloro-s-triazine

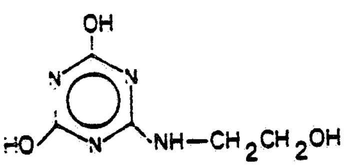


GS-10813

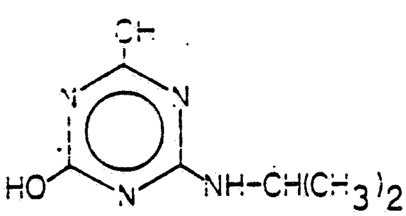
2-(Ethlyamino)-1,3,5-triazine-2,4(1H,3H)-dione

CGA-62373

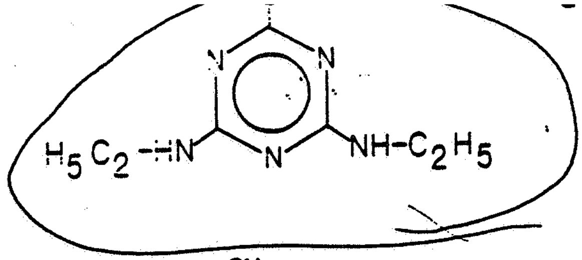


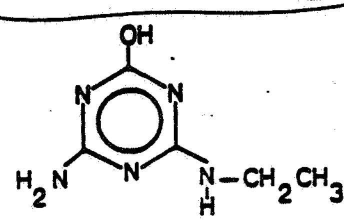


Hydroxylated sidechain metabolite of GS-10813



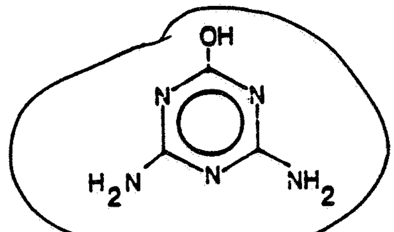
GS-11957





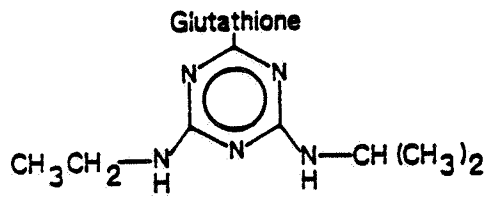
GS-17792

2-Amino-4-ethylamino-6-hydroxy-s-triazine

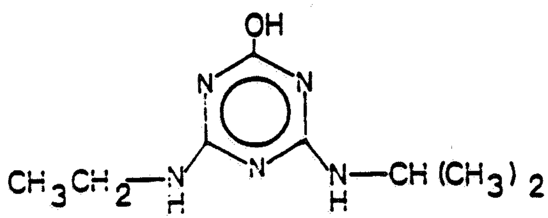


GS-17791 (Ammeline)

2,4-Diamino-6-hydroxy-s-triazine

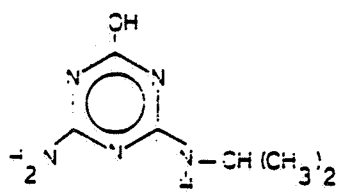


Glutathione Conjugate of Atrazine



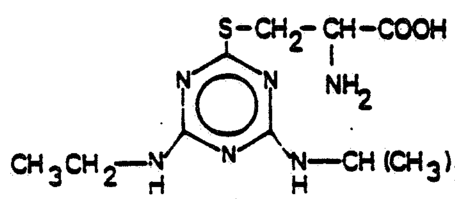
G-34048 (Hydroxy-atrazine)

2-(Ethylamino)-4-hydroxy-6-(isopropylamino)-s-triazine



GS-17794

2-Amino-4-hydroxy-6-(isopropylamino)-s-triazine



Cysteine Conjugate of Atrazine

S-[4-(Ethylamino)-6-[(1-methylethyl)amino]-1,3,5-triazin-2-yl] cysteine