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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Weight-of-the-Evidence and Oncogenic Properties of Atrazine

To: The Peer Review Committee
Toxicology Branch/HED (TS-769C)

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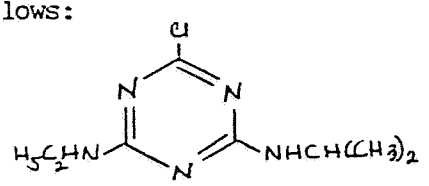
ATRAZINE

DATA EVALUATION REPORT ON ~~EXAMPLE~~ FOR
THE PEER REVIEW COMMITTEE

1. Background

Atrazine is a selective herbicide used for season-long weed control in corn, sorghum and other crops. It is used at the highest rate for non-selective weed control in noncropped areas.

The chemical name of atrazine is 2-chloro-4-ethylamino-6-isopropylamino-S-triazine. The structure is as follows:



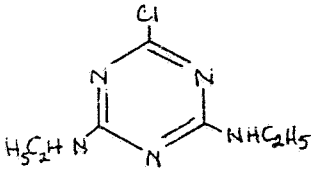
2. Metabolism

When a single oral dose of ^{14}C-atrazine was given to Long-Evans rats, 52-57% was excreted in the urine and 12-15% in the feces within 48 hours. Less than 0.1% was excreted in expired CO_2. The highest tissue residues were found in the liver and kidney. No metabolite identification was done in this study.

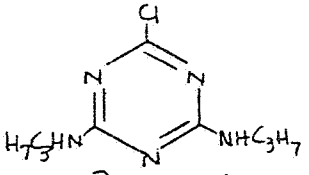
Apparently one metabolite of atrazine has been identified, a monochlorohydroxy metabolite, since a chronic dog study is being conducted on this metabolite and Ciba-Geigy has stated, in reference to this study, that it is a major metabolite. Conversation with Ciba-Geigy on August 25, 1987 indicates that this is an identified plant metabolite.

3. Structure Activity Relationship

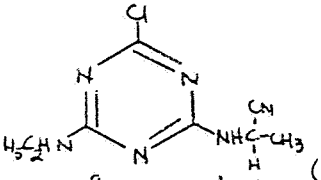
Atrazine is structurally related to simazine, cyanazine, propazine and terbutryn.



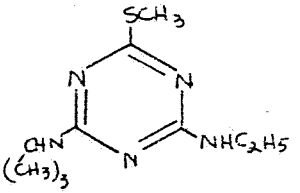
Simazine



Propazine



Cyanazine



Terbutryn

a. Simazine

Simazine is rapidly metabolized in the rat. Eighty-six percent of the labelled compound is excreted within 14 hours in the urine and feces. Oncogenicity studies are currently underway.

b. Cyanazine

In rats, 89% of labelled cyanazine is eliminated within 4 days, 42 % in urine and 47% in feces. The major metabolic pathways are dechlorination and

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deethylation. Cyanazine did not produce chromosomal aberrations in bone marrow of mice and did not appear to be oncogenic to CD mice. Adequate oncogenicity studies in the rat are not available; however, a new study in the rat is presently being conducted.

c. Propazine

Forty-two percent of ^{14}C-propazine was eliminated in the urine and 28% in the feces. Mostly unchanged propazine was found in the feces. Hydroxypropazine was identified both in urine and feces.

Propazine has been found to be positive for mutagenicity in V79 Chinese hamster cells both with and without metabolic activation. However, the response was weaker in the presence of metabolic activation. It was negative in a nucleus anomoly assay and in a DNA repair assay in rat hepatocytes.

Propazine was negative for oncogenicity in the CD-1 mouse but caused a statistically significant increase in mammary tumors in female CD rats. Propazine has recently been presented to the Toxicology Branch Peer Review Committee for classification of oncogenic potential and has been classified as a category C oncogen.

d. Terbutryn

Eighty-five percent of ring-labelled ^{14}C-terbutryn is excreted within 72 hours in the urine (39%) and feces (46%) of rats. The major metabolic pathways are desulfuration, N-deethylation and S-demethylation.

Terbutryn is not mutagenic in the Ames Salmonella assay and the micronucleus assay and does not cause chromosomal aberrations in vivo in hamsters.

Terbutryn is negative for oncogenicity in the CD-1 mouse. When administered in the diet to female Charles River CD rats, terbutryn induced a statistically significant increase in commbined mammary gland adenomas and adenocarcinomas and in combined hepatocellular adenomas and carcinomas. In males, terbutryn induced an increase in combined thyroid follicular cell adenomas and carcinomas and in testicular interstitial cell adenomas. The Toxicoogy Branch Peer Review Committee has classified terbutryn as a category C oncogen.

4. Non-oncogenic Toxicological Effects

Atrazine in Toxicity Category III for acute oral, dermal and inhalation toxicity. Atrazine is not a primary skin irritant. Conflicting results have been reported on the primary eye irritation of atrazine. One study indicates that it is nonirritating (Tox. category IV) and another indicates that that it causes corneal opacity and conjunctivitis up to and including 72 hours after application (Tox. category II).

Atrazine is not teratogenic in the rat. It has not been adequately tested for teratogenicity in another species. In a 3-generation reproduction study (supplementary), no adverse reproductive effects were seen up to 100 ppm, the highest dose tested (HDT).

In a two year dog feeding study (supplementary), the NOEL was 15 ppm and the LEL was 150 ppm based upon increased heart and liver weights in females. Recently submitted 6(a)(2) data indicates that atrazine induces gross pathological lesions in the atrium of dogs at a level of 1000 ppm. A final report of this study has not been received. Furthermore, 6(a)(2) data have also been submitted on the major metabolite of atrazine, the monochlorohydroxy, indicating that it causes atrial fibrillation in dogs at 1500 ppm after 5 weeks of treatment. The high dose level in this two year study is going to be dropped to 1000 ppm.

5. Summary of Oncogenicity Studies

a. Ciba-Geigy Rat Study

Ref.: Twenty-four month combined chronic oral toxicity and oncogenicity study in rats utilizing atrazine technical. Mayhew, DA, Taylor, GD, Smith, SH and Banas, DA. Conducted by American Biogenics Corporation for Ciba-Geigy Corp. Study No. 410-1102. Accession No. 262714-262727. April 29, 1986.

Sprague-Dawley [Crl:COBS CD(SD)BR; age 37-38 days] rats were started on diets containg either 0, 10, 70, 500 or 1000 ppm atrazine. Twenty rats per sex per group were used for the chronic toxicity group, i.e. rats used to measure blood parameters and clinical chemistries and urinalysis. Fifty rats per sex per group were used for the oncogenicity study and were maintained on diets for 24 months. An additional 10 rats per sex were placed on control and high dose diets for a twelve month interim sacrifice and another 10 per sex (control and high dose, only) for a 13 month sacrfice (the high dose group was placed on control diet for one month prior to sacrifice).

The incidence of relevant neoplastic pathology seen in this study can be found summarized in Table 1. Statistics on the mammary tumors can be found in Table 2 (taken from DER, Appendix 3) and Table 3 (taken from DER, Appendix 1).

Table 1.
Relevant Neoplastic Lesions Seen in Sprague-Dawley Rats Fed Atrazine

Organ/Neoplasm	Dosage Level (ppm)				
	0	10	70	500	1000
			Males		
Testes					
Interstitial cell tumor*	1/65(1.5)	3/65(4.6)	2/67(3.0)	2/67(3.0)	7/67(10.4)
			Females		
Mammary Gland					
adenocarcinoma	15/66(23)	15/64(23)	26/68(38)	27/65(42)	35/64(64)
fibroadenoma	29/66(44)	29/64(45)	35/68(52)	38/65(59)	42/64(66)
adenoma	1/66(1.5)	0/64(0)	1/68(1.5)	1/65(1.5)	2/64(3.1)
carcinosarcoma	0/66(0)	0/64(0)	0/68(0)	1/65(1.5)	2/64(3.1)

*Statistically significant by pairwise comparison (Fisher's exact) at 1000 ppm ($p<0.034$) and for a dose-related trend (Cochran-Armitage) at $p<0.011$.

Table 2.
Peto Prevalence Analysis[a] of Mammary Gland Adenocarcinomas
in Female Rats Dosed with Atrazine by Week of Death with Tumor

	Weeks					
Dose (ppm)	34-60[b]	Interim kill	61-78	79-105	Final kill	Total
0	0/3	0/20	1/8	8/23	6/35	15/89**
10	1/3		3/11	5/21	7/33	16/68
70	1/2		4/10	12/28	10/30	27/20**
500	0/3		3/9	13/30	11/26	27/68**
1000	2/7	6/20	7/10	20/36	8/17	43/90**

Note: Significance of trend denoted at control group. Significance of pairwise comparison denoted at dose level.
[a] Number of tumor bearing animals/number of animals at risk, excluding animals that died prior to appearance of the first tumor.
[b] Appearance of first tumor - 10 ppm group.
** $p<0.01$.

Table 3.
Statistical Analysis of Mammary Tumor Data

	Dosage Level (ppm)				
All Animals on Study	0	10	70	500	1000
No. of tissues	88	69	69	70	89
Carcinomas	15	16	27	27	45
Adenomas and fibroadenomas	29	29	36	39	46
All tumors	35	40	48	48	65
p values[a]					
Carcinomas -- Cox-Tarone			0.045	0.0071	<0.00005
-- Gehan Breslow			0.029	0.0016	<0.00005
Adenomas and fibroadenomas -- Cox-Tarone				0.0685	0.0004
All tumros -- Cox-Tarone				0.0071	<0.00005
-- Gehan Breslow				0.0050	<0.00005

[a] Life-table analyses, pairwise comparison.

Survival was adversely affected in female rats at the highest dose tested. Statistical analysis of survival rates for females can be found in Appendix 3. Survival in males was actually better than the controls at the HDT.

Table 4.
Mortality (percent survival)

Dose Group (ppm)	Males	Females
0	44	50
10	47	44
70	56	43
500	57	37
1000	67	26

Body weight was statistically significantly depressed in males and females throughout the study at 500 and 1000 ppm. Body weights can be found in Table 5 for 13 and 104 week intervals along with % decrease in body weight gain.

Table 5.
Body Weight Data at 13 and 104 Weeks

Dose Level (ppm)	B. Wt. 13 wks.	% of Control	B. Wt. 104 wks.	% of Control	% dec. B. Wt. Gain 13wks.	% dec. B. Wt. Gain 104wks.
Males						
0	532	100	704	100	0	0
10	541	102	739	105	0	0
70	516	97	742	105	5	0
500	467**	88	646	92	17	10
1000	436**	82	572**	81	25	24
Females						
0	283	100	496	100	0	0
10	282	99	477	96	2	5
70	275	97	480	97	5	5
500	252**	89	402*	81	18	25
1000	239**	84	361**	73	26	35

* Significantly different from control ($p<0.05$).
** Significantly different from control ($p<0.01$).

Several non-neoplastic lesions were induced in both males and females by atrazine. These are summarized in Table 6.

Table 6.
Relevant Non-neoplastic Lesions Seen in Male and Female Rats Fed Atrazine

Organ/Finding	Dose Level (ppm) 0	10	70	500	1000
			Males		
Mammary gland acinar hyperplasia	7/58	1/59	5/61	7/64	21/65**†
Kidney pelvic calculi	15/65	16/65	11/67	17/67	31/67**†
Prostate epithelial hyperplasia	12/65	16/63	11/66	17/67	29/66**†
Muscle-rectus femoris degeneration	6/64	7/65	7/67	10/66	28/67**†
			Females		
Bone Marrow - femur myeloid hyperplasia	25/68	23/65	24/69	38/65	52/64**†
Bone Marrow - sternum myeloid hyperplasia	21/68	21/65	20/69	33/65*	46/64**†
Spleen extramedullary hematopoiesis	12/67	14/65	18/69	22/65*	28/65**†
Kidney hyperplasia, transitional epithelium	17/68	10/65	5/68	19/65	31/65**
Urinary Bladder hyperplasia, transitional epithelium	4/67	0/65	1/69	3/65	10/64*
Muscle - rectus femoris degeneration	5/67	4/65	9/69	8/64	13/64*†
Eye retinal degeneration	12/68	9/65	13/69	16/65	22/65**
Liver centrolobular necrosis	3/68	3/65	1/69	4/65	12/65**

* Significantly different from control ($p<0.05$).
** Significantly different from control ($p<0.01$).
† Positive dose-related trend ($p<0.01$).

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b. IARC Rat Study (DER Appendix 2).

A chronic feeding/oncogenicity study was conducted for IARC on atrazine in Fischer 344/LATI rats at the Hungarian Institute of Hygiene. Dosages used were 0, 375 and 750 ppm. Only preliminary results are available from this study and according to Ciba-Geigy the final report most likely will not be made available to them.

Available information indicates the following relevant tumor types were seen in this study.

Table 7.
Relevant Tumor Types - Fischer 344 Study

Dosage Level (ppm)	Leukemia/lymphoma	benign mammary tumors
Males		
0	22/47	1/48
375	27/47	1/51
750	32/48	9/53*
Females		
	leukemia/lymphoma	uterine adenocarcinomas
0	12/44	7/45
375	16/52	10/52
750	22/51**	14/45**

* includes 1 carcinoma. Statistically significant with Peto's incidental tumor test.
** Statistically significant for dose-related trend (Cochran-Armitage).

6. Historical Control Information

Historical control information from the performing laboratory can be found summarized in Table 8 for four different studies.

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Table 8.
Historical Control Data for Testicular and Mammary Tumors

Organ/Tumor Type	Study Number A	B	C	D
		Males		
Testis				
interstitial cell tumors	0/44 (0)	5/90 (6)	3/80 (4)	7/60 (12)
		Females		
Mammary gland				
fibroadenoma	17/45 (38)	43/90 (48)	29/80 (36)	24/58 (41)
adenocarcinoma	4/45 (9)	17/90 (19)	3/80 (4)	2/58 (3)
adenoma	0/45 (0)	0/90 (0)	1/80 (1)	4/58 (7)
carcinosarcoma	0/45 (0)	0/90 (0)	0/80 (0)	0/58 (0)
tumor bearing	18/45 (40)	46/90 (51)	32/80 (40)	28/58 (48)

Range Interstitial Cell Tumors 0 - 12 %
Range Mammary Gland Tumors
fibroadenomas 36 - 48 %
adenocarcinoma 3 - 19 %
carcinosarcoma 0 %
tumor-bearing 28 - 51 %

7. Mutagenicity

a. Ames Salmonella assay: negative in an acceptable assay.
b. Rec-assay and reversion assay: negative in an acceptable assay in Bacillus Subtilis, strains H17 Rec^+ and M45 Rec^-.
c. Nuclear Anomaly Assay (micronucleus) in Chinese Hamsters: negative in an unacceptable assay.
d. Unscheduled DNA Synthesis Assay in Human Fibroblast: negative in an unacceptable assay.
e. Unscheduled DNA Synthesis Assay in Rat Hepatocytes: reported negative in a recently submitted, unreviewed assay.
f. Dominant Lethal Assay in Mice: negative but unacceptable. Registrant recently submitted arguments as to its acceptability, as yet unreviewed.

8. Summary

Administration of atrazine to rats resulted in an increased incidence of mammary gland tumors in females and testicular interstitial cell tumors in males. The incidence of fibroadenomas and adenocarcinomas exceeded the historical control range in this strain (Sprague-Dawley) of rats. The incidence of testicular tumors was within the historical control range. An acceptable study in the mouse is not available. Atrazine was negative for mutagenicity in a battery of tests. Atrazine is structurally related to simazine, cyanazine, propazine and terbutryn. Both propazine and terbutryn have been classified as Category

oncogens by the Toxicology Branch Peer Review Committee. They both induce mammary gland tumors in rats and terbutryn also induces testicular tumors in rats. Atrazine was not teratogenic to rats and its administration resulted in no reproductive toxicity.