7/31/89

FILE COPY

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

JUL 31 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Simazine

FROM: Esther Rinde, Ph.D. E. Rinde 6/16/89
Science Analysis and
Coordination Branch
Health Effects Division (TS-769c)

TO: James Yowell
Product Manager #23
Registration Division (TS-767c)

The Health Effects Division Peer Review Committee met on May 17, 1989 to discuss and evaluate the weight-of-the-evidence on Simazine with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp	Penelope A. Fenner-Crisp
William L. Burnam	William L. Burnam
Reto Engler	Reto Engler
Edwin R. Budd	Edwin R. Budd
Marcia Van Gemert	Marcia Van Gemert
Karl Baetcke	Karl P. Baetcke
Marion Copley	Marion P. Copley
Kerry Dearfield	Kerry Dearfield
Richard Levy	Richard Levy

A. 1. Peer Review Committee (contd.)

John Quest

Esther Rinde

William Sette

Lynnard Slaughter

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Henry Spencer

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Richard Hill

Robert Beliles

George Ghali

4. Other Attendees:

Esther Saito (HED) was also present.

B. Material Reviewed:

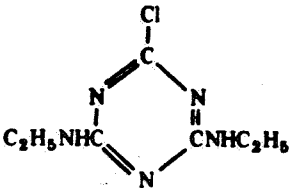
The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. Henry Spencer; tables and statistical analysis by Dynamac. The material reviewed is attached to the file copy of this report.

C. Background Information:

Simazine is one of several triazine compounds which are used in agriculture as herbicides to control annual grasses and broadleaf weeds in corn, alfalfa, orchards of cherries, peaches, citrus, apples, pears and asparagus as well as ornamentals and nursery stock. Simazine is also registered for use in controlling algae in ponds. Little of the Simazine parent chemical is found as residues in food and feed crops.

Following the Data-Call-In Notice of the first Registration Standard of 1984, new chronic toxicity studies were received; these were evaluated by the Onco Peer Review Committee.

Structure of Simazine:



Simazine▼

D. Evaluation of Oncogenicity Evidence for Simazine:

1. CD-1 Mouse Oncogenicity Study

Reference: Hazelette, JR and JD Green: "Simazine Technical; 95-week Oral Toxicity/Oncogenicity Study in Mice.", April 4, 1988. Accession/MRID Number: 406144-04, Lab. Study Number: 842121. Testing Facility: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

Simazine technical was administered in the diet to groups of 60 male and 60 female Crl:CD1(ICR)BR mice at 0 (control), 40, 1000 or 4000 ppm for 95 weeks.

There were no increases in neoplasms reported for any dosed group.

There was no evidence of a compound-related effect on survival or target organ toxicity.

The dosing was considered to be adequate for assessing the oncogenic potential of Simazine, based on body weight gain depressions of 14% in males and 19% in females seen at 1000 ppm.

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study

Reference: McCormick, CC and AT Arthur: "Simazine-Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats." , April 12, 1988. MRID Number: 406144-05. Study Number: 2-0011-09. Testing Facility: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

Simazine technical was administered in the diet to groups of 50 male and 50 female rats at 0 (control), 10, 100 or 1000 ppm for 2 years. Additional groups (30-40/sex/dose) were also treated.

> In female rats there was a statistically significant increase in mortality, and in male rats there was a statistically significant decrease in mortality, with increasing doses of Simazine.

Neoplastic lesions which occurred with statistically significant increases were reported as follows:

In female rats, there was a statistically significant dose-related trend ($p<.01$) for mammary gland carcinomas and combined adenomas/fibromas/carcinomas; however, when the shortened life-span of the female rats was included in the statistical evaluation, the incidences of carcinoma alone at both the 100 and 1000 ppm (HDT) dosage groups were statistically significantly increased as well ($p<.05$ and $p<.01$, respectively). The upper limit of the historical control incidence reported for mammary carcinoma (Table 1) was exceeded at 100 ppm, and greatly exceeded at 1000 ppm (HDT). The incidence of cystic glandular hyperplasia in the mammary gland was statistically significantly increased at the HDT, which correlates with the observed high tumor incidence at that dose.

There was a statistically significant dose-related trend for kidney tubule adenomas ($p<.05$); however (as in the case of the male rats) tumors occurred only at the HDT and the incidence (3.6%) was not statistically significant by pairwise comparison with that in the concurrent control. The incidences for adenomas and/or carcinomas reported for historical female controls (Table 1) were zero in all 7 studies (Table 1).

TABLE 1

HISTORICAL CONTROL TUMORINCIDENCE DATA
NUMBER OF TUMOR-BEARING ANIMALS - SPRAGUE-DAWLEY RATS

Submitted by Ciba-Geigy

COMPOUND	83 A	JAN 83 B	NOV 83 C	84 D	85 E	85 F	85 G
SITE: NEOPLASM	NUMBER OF NEOPLASMS						
MAMMARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	6	6	8	2	5	3	2
FIBROADENOMA	18	16	26	21	12	23	22
ADENOMA/FIBROADENOMA (COMBINED)	22	18	30	22	15	25	23
ADENOCARCINOMA	7	4	5	11	9	15	14
ALL MAMMARY TUMORS (COMBINED)	25	22	34	30	20	34	32
PITUITARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(63)	(60)	(69)	(69)	(60)	(70)	(70)
ADENOMA	52	49	55	59	49	62	62
CARCINOMA	0	2	2	2	6	2	1
ADENOMA AND CARCINOMA (COMBINED)	52	51	57	61	55	64	63
KIDNEY (MALES AND FEMALES):							
NUMBER OF SITES EXAMINED	(65/65)	(60/59)	(70/70)	(70/70)	(60/60)	(70/70)	(70/70)
	M F	M F	M F	M F	M F	M F	M F
ADENOMA	0 0	0 0	2 0	1 0	0 0	0 0	0 0
CARCINOMA	0 0	0 0	0 0	1 0	0 0	0 0	0 0
ADENOMA AND CARCINOMA (COMBINED)	0 0	0 0	2 0	2 0	0 0	0 0	0 0
ADRENAL GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	1	3	4	2	3	2	8
LIVER (MALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	0	2	0	2	10	4	1
CARCINOMA	0	1	1	6	2	1	0

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study (contd.)

In female rats, there were also statistically significant dose-related trends for adenomas, carcinomas and combined adenoma/carcinomas of the pituitary gland ($p<.01$). Pairwise comparisons were significant only for carcinomas at 1000 ppm ($p<.05$) and only when time adjusted, assuming fatal tumor context, to account for the effect of mortality disparity in the animals (the mortality in female rats was statistically significantly increased compared to controls at 100 and 1000 ppm). The incidence of pituitary gland carcinoma at 1000 ppm (HTD) only slightly exceeded the upper bound of the historical control range; however, it greatly exceeded the incidence reported in 6 out of 7 studies.

> Tables 4, 5 and 6 (from the Dynamac "..Qualitative Risk Assessment...." 10/18/88, attached) summarize these findings; a fatal tumor analysis was performed on the female rat pituitary gland tumors, as described on pg. 8 of that memo.

Historical control tumor incidence data for Sprague-Dawley rats at the testing facility are given in Table 1.

In male rats, the incidences of liver tumors were statistically significantly increased for carcinoma and for combined adenoma/carcinoma at 100 ppm and 1000 ppm (HDT), respectively ($p<.05$); however, these incidences fell within the range reported for historical controls at the testing facility.

There was also a statistically significant dose-related trend for kidney tubule carcinomas ($p<.05$), and for combined adenoma/carcinoma ($p<.01$); however, tumors occurred only at the HDT and neither the carcinoma (3%) nor the combined adenoma/carcinoma (5%) incidence was statistically significant by pairwise comparison with that in the concurrent control (2% in both cases).

> Tables 7 and 9 (from the attached Dynamac memo) present data for the tumor incidences (adjusted for mortality differences) in liver and kidney, respectively. The rationale for the tumor analysis is presented on page 8 of the Dynamac memo.

Table 4. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Mammary Gland Tumor Rates+ and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma					
Fibroadenoma	23/89	20/78a	11/71	21/75	
	(26)	(26)	(15)	(28)	(27-37)
	p= 0.0689	p= 0.302	p= 0.177	p= 0.123	
Carcinoma	16/89	13/80	20/75b	40/78	
	(18)	(16)	(27)	(51)	(7-21)
	p< 0.0001**	p= 0.4740	p= 0.0392*	p< 0.0001**	
Adenoma					
Carcinoma	39/89	33/80	31/75	61/78	
	(44)	(41)	(41)	(78)	
	p< 0.0001**	p= 0.4064	p= 0.2229	p< 0.0001**	

a First Adenoma observed at 48 weeks in dose 10 ppm and the first Fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.

b First carcinoma observed at 48 weeks in dose 100 ppm.

Table 5. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Kidney Tubule Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Controls
Adenoma	0/74	0/62	0/54	2/55c	
	(0.0)	(0.0)	(0.0)	(3.6)	(all 0)
	p= 0.0042**	p= 1.0000	p= 1.0000	p= 0.1799	

c First Adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were coded.

+ Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animal not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TABLE 6. SIMAZINE, SPRAGUE-DAWLEY RAT Study--FEMALE Pituitary Gland Tumor Rates+, Fatal Tumor Analysis and Generalized K/W Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma	73/89	57/80	63/77 a	61/79	
	(82.0)	(71.2)	(81.8)	(77.2)	(80-89)
	p= 0.0033**	p= 0.9944	p= 0.0206*	p= 0.0030**	
Carcinoma	1/73	3/61	0/52	6/53 b	
	(1.4)	(4.9)	(0.0)	(11.3)	(0-10)
	p= 0.0010**	p= 0.2351	p= 0.4545	p= 0.0153*	
Adenoma Carcinoma	74/89	60/80	63/77	67/79	
	(83.1)	(75.0)	(81.8)	(84.8)	(83-92)
	p= 0.0005**	p= 0.8351	p= 0.0251*	p=0.0005**	

\+ Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the first tumor or animals not examined).

( ) Per cent

a First Adenoma observed at 35 weeks in dose 100 ppm.

b First Carcinoma observed at 72 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p > 0.01$

Table 7. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Liver Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma	1/88 (1.1)	2/79a (2.5)	0/80 (0.0)	3/80 (3.8)	(0-17)
	p= 0.0824	p= 0.4594	p= 0.5238	p= 0.2752	
Carcinoma	0/88 (0.0)	2/79 (2.5)	4/80b (5.0)	3/80 (3.8)	(0-9)
	p= 0.2169	p= 0.2223	p= 0.0494*	p= 0.1058	
Adenoma Carcinoma	1/88 (1.1)	4/79 (5.1)	4/80 (5.0)	6/80 (7.5)	
	p= 0.0643	p= 0.1519	p= 0.1554	p= 0.0449*	

a First Adenoma observed at 52 weeks in dose 10 ppm.
b First Carcinoma observed at 99 weeks in dose 100 ppm.

+ Number of tumor bearing animals/Number of animals at risk (excluding animals that died before 52 weeks or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Table 9. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Kidney Tubule Tumor Rates+ and Peto Prevalence Test Results-

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma	0/51	0/46	0/48	1/57a	
	(0)	(0)	(0)	(2)	(0-3)
	p= 0.0543	p= 1.0000	p= 1.0000	p= 0.5278	
Carcinoma	1/66	0/62	0/64	2/65b	
	(2)	(0)	(0)	(3)	(0-1)
	p= 0.0332*	p= 0.1660	p= 0.1821	p= 0.2091	
Adenoma Carcinoma	1/66	0/62	0/64	3/65	
	(2)	(0)	(0)	(5)	(0-3)
	p= 0.0056**	p= 0.1410	p= 0.1721	p= 0.1087	

a First Adenoma observed at 92 weeks in dose 1000 ppm.

b First Carcinoma observed at 78 weeks in dose 1000 ppm

c The p values for Adenomas were calculated using the Cochran-Armitage Trend Test and Fisher's Exact Test, since the Peto Prevalence method collapsed to one interval.

+ Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study (contd.)

The Committee agreed that the highest dose exceeded the MTD for female rats, based on excess deaths and body weight gain reductions of 28-45% (days 7-728). The highest dose in males appeared to have exceeded the MTD, as well, based on body weight gain reductions of 27-36% (days 7-728). The Committee also felt that there was too great an interval between the mid and high doses (100 to 1000 ppm).

E. Additional Toxicology Data on Simazine:

1. Metabolism

Simazine exhibits increased binding affinity for red blood cells following oral dosing in the rat. Almost all of orally administered Simazine was excreted in the feces and urine 96 hours after administration to rats.

2. Mutagenicity

Three mutagenicity tests have been submitted in support of the registration for Simazine. Simazine was negative in an acceptable Salmonella assay using strains TA98, TA100, TA1535, TA1537 and TA1538, with and without activation. The other two tests were found to be unacceptable: a cytogenetics assay with cultured human lymphocytes and an unscheduled DNA synthesis (UDS) assay with primary rat hepatocytes. Therefore, of the three categories of mutagenicity testing, only the gene mutation category is minimally fulfilled with data gaps in the structural chromosomal aberrations and other genotoxic effects categories.

The negative Salmonella results are consistent with published literature and results with other s-triazine herbicides. However, it is reported in the literature that Simazine is positive for gene mutations in the mouse lymphoma assay (Waters et al., Basic Life Sci 21: 275-326, 1982), the Drosophila sex-linked recessive lethal assay (ibid; also reported by the U.S. EPA Gene-Tox Program), cell transformation in Syrian hamster embryo cells (reported by the U.S. EPA Gene-Tox Program), and plant cytogenetic assays (for review see Plewa et al., Mutat Res: 136 233-245, 1984). Simazine was also reported in the literature as being negative in several other assays including yeast assays, UDS with a human cell strain, sister chromatid exchanges and a mouse micronucleus (an unacceptable protocol) (Waters et al., 1982). It was also reported negative in two assays for aneuploidy (see Dellarco et al., Mutat Res 167: 149-169, 1986).

E. 2. Mutagenicity (contd.)

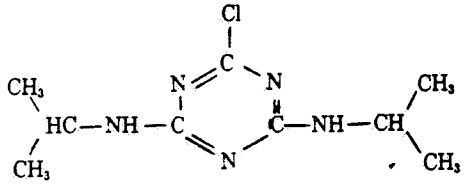
It appears then that Simazine has genotoxic potential and this would provide some support for an oncogenicity concern. Tests for submission to satisfy data gaps and to examine in more detail this genotoxic potential should include a mouse lymphoma assay, an in vivo micronucleus test and a cell transformation assay.

3. Developmental Toxicity

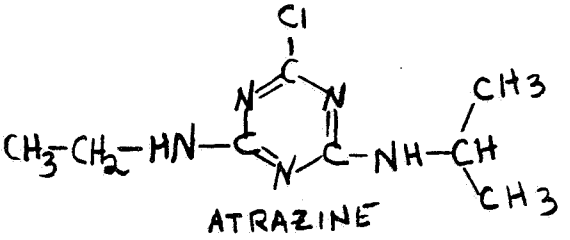
Simazine did not produce terata in the rat, when given by gavage at doses up to 600 mg/kg or in the rabbit at doses up to 200 mg/kg, by gavage; however, maternal toxicity and fetotoxicity (incomplete ossification) were observed in both species.

4. Structure-Activity Correlations

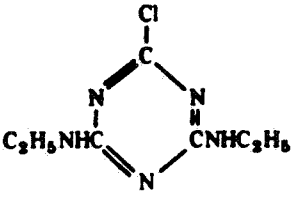
Simazine is structurally related to Atrazine, Propazine, Cyanazine, Ametryn and Prometryn. Atrazine was associated with increased mammary gland tumors in the female albino rat and was categorized as a "C(q)" oncogen by the HED Peer Review Committee. Propazine was also associated with increased mammary gland tumors in the female CD-1 rat and was categorized by the Committee as a "C" oncogen. Ametryn, Prometryn and Cyanazine have not yet been evaluated.



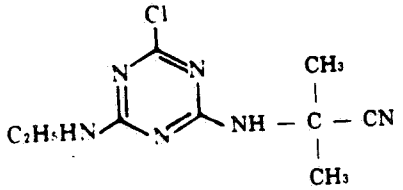
Propazine▼

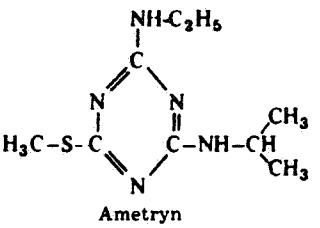


ATRAZINE

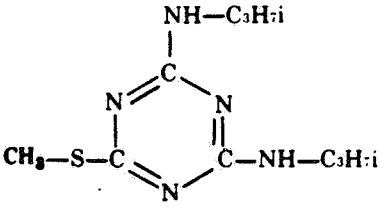


Simazine▼





Ametryn



F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Simazine to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Simazine was not associated with increases in neoplasms when fed in the diet to CD-1 mice, at doses up to 4000 ppm. The study was considered to have been adequately conducted.

2. Simazine was associated with statistically significant increases in carcinomas of the pituitary gland (at the HDT) and mammary gland (at the mid (100 ppm) and highest dose) in the female Sprague-Dawley rat, when fed in the diet at doses up to 1000 ppm. The incidence of mammary gland tumors at the HDT was well outside the range reported for historical controls at the testing facility. The incidence of pituitary gland tumors was just outside the historical control range; however, it exceeded (considerably) the incidences reported for 6 out of 7 studies.

3. The pituitary tumors in the female rats were fatal with a possibly accelerated onset, and the mammary carcinomas also contributed to the increased mortality at the HDT, according to the study authors.

4. Although the HDT may have exceeded the MTD, the mid-dose was well below, and the mammary tumors in the female rat were statistically significantly increased at both the mid and high dose. There was also too great an interval between the mid and high doses: 100 and 1000 ppm, respectively.

5. While a hormonal influence was suggested based on the pituitary and mammary gland tumors, supporting evidence was not presented.

6. There was some evidence of genotoxicity.

7. The mammary tumor response is consistent with that seen with other triazines. Both Atrazine and Propazine, triazines with structures closely related to Simazine, were associated with mammary gland tumors in the female rat.

F. Weight of Evidence (contd.)

8a. The incidence of kidney tubule adenomas at the HDT in the female rat, although not statistically significant, exceeded that reported for historical controls (zero) in all seven studies at the testing facility. While this tumor incidence fits the NTP definition of a "rare" tumor (≤1% incidence), Dr. Slaughter offered, that based on his experience, the historical incidence of rat kidney tumors is more accurately defined as "uncommon").

8b. The incidence of kidney tubule carcinomas in male rats was less clearly defined (because of sporadic occurrences of the same tumor in control animals).

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Committee evaluated all of the evidence listed in part F (above) and concluded that Simazine should be classified as a Category C Oncogen (possible human carcinogen), based on evidence in one species, one sex. The Committee also called for a quantitative risk assessment for Simazine, quantification to be based on the mammary tumors in the female rat. The arguments for quantification were given as follows:

1a. The tumors in both the pituitary and mammary glands of the female rat were malignant.

1b. Pituitary tumors in female rats were fatal with a possible accelerated onset (analysis to be provided).

2a. Mammary tumors were statistically increased at 2 doses, albeit one above the MTD; however, there was too large a spread between the mid and high doses.

2b. Evidence of progression was suggested by mammary hyperplasia at the HDT, which correlated with tumors at that dose.

3. There was no supporting evidence for demonstrating an hormonal influence.

4. There was equivocal evidence of kidney tumors ("rare" or at least "uncommon" tumor type) in both sexes.

5. SAR was strongly supportive. Other closely-related triazines (Atrazine and Propazine) were also associated with mammary gland tumors in the female rat.

6. There was some evidence of genotoxicity.