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SIMAZINE - Qualitative Risk Assessment from a Rat Two Year Oral Chronic Toxicity and Oncogenicity Study

Caswell No. 740

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SUMMARY:

Simazine technical was fed to male and female Sprague-Dawley rats at doses of 0, 10, 100, or 1000 ppm in a 104 week chronic toxicity/oncogenicity study.

For female rats, there was a statistically significant increase in mortality with increasing doses of Simazine and mortality was significantly increased in both the 100 and 1000 ppm dose groups compared to the controls.

The incidence of mammary gland carcinomas and combined adenomas and carcinomas had a significant dose-related trend. The incidence of mammary gland carcinomas was significantly increased compared to the controls at the 100 and the 1000 ppm groups; the combined adenomas and carcinomas was significantly increased compared to the controls for the 1000 ppm group.

The pituitary gland tumors were considered fatal (reference page 1460 of the Ciba-Geigy report, attached), all three tumor groups (adenomas, carcinomas, and combined adenomas and carcinomas) showed significant dose-related trends. The incidence of pituitary adenomas and combined tumors was significantly increased compared to controls at the 100 and 1000 ppm groups; the incidence of carcinomas was significant at the 100 ppm group only.

There was a significant dose-related trend for kidney tubule adenomas.

For male rats, there was a statistically significant decrease in mortality with increasing doses of Simazine and mortality was significantly decreased in the 1000 ppm group compared to the controls.

There were no significant dose-related trends for liver adenomas, carcinomas, and combined adenomas and carcinomas. The incidence of liver carcinomas in the 100 ppm group was significantly increased compared to the controls. The incidence of combined liver adenomas and carcinomas was significantly increased compared to the controls in the 1000 ppm group. There were no significant dose-related trends or pair-wise differences for thyroid C-cell adenomas, carcinomas, and combined adenomas and carcinomas.

There was a significant dose-related trend for kidney tubule carcinomas and combined adenomas and carcinomas. There were no significant pair-wise differences for any of the kidney tubule tumors.

BACKGROUND:

Simazine technical was fed to male and female Sprague-Dawley rats at doses of 0, 10, 100, or 1000 ppm in a 104 week chronic toxicity/carcinogenicity study. Approximately 10 animals in each sex were sacrificed after 52 weeks of continuous dosing in each dose group. Only 9 animals were sacrificed in the male 10 ppm dose group and in the female 100 and 1000 ppm dose groups. This was due to deaths on study which occurred before the scheduled sacrificed since the animals to be sacrificed were selected prior to the beginning of the study. Also ten animals from the 1000 ppm group are not included in this analysis. These animals were dosed for 52 weeks and then maintained for 52 additional weeks on an untreated (control) diet. They were designated as a recovery group. A supplementary table of the results from these animals and their assigned controls was prepared (attachment 1). There were only 2 kidney tumors in the males, one adenoma in the control group and one carcinoma in the 1000 ppm group. In the females, there were 4 mammary gland adenomas in the controls and 2 in the 1000 ppm group. There was 1 mammary gland carcinoma in the controls and 4 in the 1000 ppm group. There were no pituitary gland carcinomas in either group but there were 9 adenomas in both groups.

The study was conducted by Ciba-Geigy Corporation, Pharmaceutics Division, Summit, NJ for the Ciba-Geigy Corporation. The TOX Chemical No. is 740, the MRID No. is 406144-05, and the Study No. is 2-011-09. Data was extracted from a final report dated April 12, 1988. Test animals were assigned randomly to the following dose groups:

Table 1. Experimental Design for Rat Chronic/Carcinogenicity Study

Dose (ppm)	Phase	Total Number		Time of Sacrifice 52 Weeks		Least Number of Dose Weeks
		Male	Female	Male	Female	
Control	Chronic c	10	10	10	10	52
		10	10			52 + 52-wk recovery
		20	20			104
	Carcinogenicity	50	50			104
10	Chronic c	10	10	10a	10	52
		20	20			104
	Carcinogenicity	50	50			104
100	Chronic c	10	10	10	10a	52
		20	20			104
	Carcinogenicity	50	50			104
1000	Chronic c	10	10	10	10a	52
		10b	10b			52 + 52-wk recovery
		20	20			104
	Carcinogenicity	50	50			104

a Only 9 animals were actually sacrificed in these dose groups.
b These 10 animals were excluded from analysis.
c The chronic animals were also used for hematology, biochemistry, and urinalysis.

SURVIVAL ANALYSIS:

In female rats, a statistically significant increasing trend in mortality was observed with increasing doses of Simazine ($p = 0.0036$). Mortality was significantly increased in the 100 ppm and the 1000 ppm dose group compared to the controls ($p = 0.0058$ and $p = 0.0006$ respectively). (Table 2).

In male rats, a statistically significant decreasing trend in mortality was observed with increasing doses of Simazine ($p = 0.0016$). Mortality was significantly decreased in the 1000 ppm dose group compared to the controls ($p = 0.0077$) (Table 3) .

Tests for mortality were made using the Thomas, Breslow, and Gart procedure. The earlier deaths occurred in the mid and high dose groups and the F/W test gives more weight to earlier deaths. Hence, all mortality test reported are the generalized K/W test.

TABLE 2. SIMAZINE, SPRAGUE-DAWLEY RAT Study--FEMALE Mortality Rates* and Generalized K/W Test Results

DOSE(PPM)	1-26	27-52	WEEKS 52a	53-78	79-106a	TOTAL
0.000	0/90 (0)	1/90 (1)	10/10	13/79 (16)	39/66 (59)	53/80** (66)
10.000	0/80 (0)	2/80 (2)	10/10	18/68 (26)	27/50 (54)	47/70 (67)
100.000	1/80 (1)	8/79 (10)	9/9	18/62 (29)	26/44 (59)	53/71** (75)
1000.000	0/80 (0)	5/80 (6)	9/9	21/66 (32)	31/45 (69)	57/71** (80)

TABLE 3. SIMAZINE, SPRAGUE-DAWLEY RAT Study--MALE Mortality Rates* and Generalized K/W Test Results

DOSE(PPM)	1-26	27-52	WEEKS 52a	53-78	79-106a	TOTAL
0.000	0/90 (0)	2/90 (2)	10/10	12/78 (15)	34/66 (52)	48/80** (60)
10.000	0/80 (0)	1/80 (1)	9/9	8/70 (11)	38/62 (61)	47/71 (66)
100.000	0/80 (0)	0/80 (0)	10/10	6/70 (9)	33/64 (52)	39/70 (56)
1000.000	0/80 (0)	0/80 (0)	10/10	6/70 (9)	22/64 (34)	28/70** (40)

* Number of animals that died during the interval/Number of animals alive at the beginning of the interval.

( ) Per cent

a Interim sacrifice was conducted at 52 weeks. Final sacrifice occurred at week 106.

Note: Time intervals were selected for display purposes only. Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p > 0.01$

TUMOR ANALYSIS:

Due to the presence of mortality differences in both sexes of rats, the Peto prevalence test was used for incidental tumor rates to test for increasing incidence with increasing dose levels and for pair-wise differences between controls and treated rats. If the Peto prevalence method reduces to too few intervals then the Cochran-Armitage method is used to test for trends and the Fisher's exact test to test for pair-wise differences. If the tumors are considered fatal, the Thomas, Breslow, and Gart procedure is used to analyze for trends and pair-wise differences.

In the female rats, M. Copley suggested that the mammary gland adenomas and fibroadenomas be analyzed together as benign tumors, since about 50% of the rats with fibroadenomas also had carcinomas. There were no significant pair-wise comparisons or a trend noted. There was a significant dose-related trend for mammary gland carcinomas and for combined mammary gland adenomas/fibroadenomas and carcinomas ($p < 0.0001$). The incidence of mammary gland carcinomas in the 100 ppm and 1000 ppm dose groups were significantly increased ($p = 0.0392$ and $p < 0.0001$, respectively) compared to the controls. The incidence of combined mammary gland adenomas/fibroadenomas and carcinomas in the 1000 ppm dose group was significantly increased ($p < 0.0001$) compared to the controls (Table 4).

There was a significant dose-related trend for kidney tubule adenomas ($p = 0.0042$) by the Cochran-Armitage trend test (Table 5). The Cochran-Armitage trend test was used since the Peto prevalence procedure reduced to one interval. There were no significant pair-wise differences found using the Fisher's exact test for pair-wise differences.

A fatal tumor analysis was performed on female rat pituitary gland tumors (reference page 1460 of the Ciba-Geigy report, attached) and the generalized K/W analysis test results reported. There was a significant dose-related trend for pituitary gland adenomas only, carcinomas, and combined adenomas and carcinomas ($p = 0.0033$, $p = 0.0010$, and $p = 0.0005$ respectively) (Table 6). The incidence of pituitary gland adenomas in the 100 ppm and the 1000 ppm dose group was significantly increased ($p = 0.0206$ and $p = 0.0030$ respectively). The incidence of pituitary gland carcinomas was significantly different from the controls in the 1000 ppm dose group ($p = 0.0153$). The 100 ppm and 1000 ppm dose group of combined pituitary adenomas and carcinomas was significantly different from the controls ($p = 0.0251$ and $p = 0.0005$ respectively).

From an examination of the Kaplan-Meier survival curves (copies available), the pituitary adenoma/carcinoma lesions appear 4 to 15 weeks earlier in the 100 ppm and 1000 ppm dose

groups than they do in the 10 ppm dose or control groups. The incidence of the mid and high group remain higher than the other two groups until near the end of the study.

In the male rats, there were no dose-related trends for liver adenomas, carcinomas, or combined liver adenomas and carcinomas by the Cochran-Armitage trend test (Table 7). The incidence of liver carcinomas in the 100 ppm group was significantly increased ($p = 0.0494$) compared to the controls by the Fisher exact test. The incidence of combined liver adenomas and carcinomas in the 1000 ppm group was significantly increased ($p = 0.0449$) compared to the controls. The Cochran-Armitage trend test and the Fisher's exact test were used because only one interval was calculated using the Peto prevalence test. For the liver carcinomas, animals that died before 52 weeks were excluded from analysis, although the first carcinoma appears at week 99. It was assumed that 52 weeks was an adequate time period for liver tumors to appear.

There were no significant pair-wise differences or dose-related trends for thyroid C-cell adenomas, carcinomas or combined thyroid C-cell adenomas and carcinomas (Table 8).

There was a significant dose-related trend for kidney tubule carcinomas and combined kidney tubule adenomas and carcinomas ($p = 0.0332$ and $p = 0.0056$, respectively) (Table 9). There were no significant pair-wise differences between treated groups and the controls for kidney adenomas, carcinomas or combined adenomas and carcinomas. Analysis of kidney tubule adenoma was done with the Cochran-Armitage trend test and Fisher's exact test since the Peto prevalence procedure resulted in only one interval.

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
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p= 0.0689	p= 0.302	p= 0.177	p= 0.123
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p< 0.0001**	p= 0.4740	p= 0.0392*	p< 0.0001**
Adenoma Carcinoma	39/89 (44)	33/80 (41)	31/75 (41)	61/78 (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
Adenoma	0/74 (0.0)	0/62 (0.0)	0/54 (0.0)	2/55c (3.6)
	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
Adenoma	73/89	57/80	63/77 a	61/79
	(82.0)	(71.2)	(81.8)	(77.2)
	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)
	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)
	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
Adenoma	1/88	2/79a	0/80	3/80
	(1.1)	(2.5)	(0.0)	(3.8)
	p= 0.0824	p= 0.4594	p= 0.5238	p= 0.2752
Carcinoma	0/88	2/79	4/80b	3/80
	(0.0)	(2.5)	(5.0)	(3.8)
	p= 0.2169	p= 0.2223	p= 0.0494*	p= 0.1058
Adenoma Carcinoma	1/88	4/79	4/80	6/80
	(1.1)	(5.1)	(5.0)	(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 8. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Thyroid C-Cell Tumor Rates+ and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	2/52	7/52a	5/51	6/58
	(4)	(13)	(10)	(10)
	p= 0.3355	p= 0.0606	p= 0.1082	p= 0.0870
Carcinoma	2/34	1/31	1/36	3/45b
	(6)	(3)	(3)	(7)
	p= 0.1762	p= 0.1082	p= 0.2881	p= 0.4183
Adenoma Carcinoma	4/52	8/52	6/51	9/58
	(8)	(15)	(12)	(16)
	p= 0.1924	p= 0.1965	p= 0.2261	p= 0.1505

a First Adenoma observed at 89 weeks in dose 10 ppm.

b First Carcinoma observed at 102 weeks in dose 1000 ppm.

+ 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 $p < 0.01$

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
Adenoma	0/51 (0)	0/46 (0)	0/48 (0)	1/57a (2)
	p= 0.0543	p= 1.0000	p= 1.0000	p= 0.5278
Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	2/65b (3)
	p= 0.0332*	p= 0.1660	p= 0.1821	p= 0.2091
Adenoma Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	3/65 (5)
	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 $p < 0.01$

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ATTACHMENT 1. SIMAZINE Female Rat Tumor Rates in rats fed 1000 ppm fo 52 weeks and then allowed a 52-week recovery period compared to their matching control groups.

Tumor	Dose(ppm)	0	1000	0	1000
		FEMALES		MALES	
Mammary Gland:					
Adenoma and/or Fibroadenoma		4/10	2/10		
Carcinoma		1/10	4/10		
Adenoma/Fibroadenoma/ Carcinoma Combined		5/10	6/10		
Pituitary:					
Adenoma only		9/10	9/10		
Carcinoma		0/10	0/10		
Adenoma and/or Carcinoma		9/10	9/10		
Kidney Tubules:					
Adenomas		0/10	0/10	0/10	1/10
Carcinoma				1/10	0/10
Adenoma and/or Carcinoma				1/10	1/10
Liver:					
Adenoma only				0/10	0/10
Carcinoma				0/10	0/10
Adenoma and/or Carcinoma				0/10	0/10
Thyroid C-Cell:					
Adenomas				0/10	0/10
Carcinoma				0/10	0/10
Adenoma and/or Carcinoma				0/10	0/10

SIMAZINE Female Rat Tumor Rates:

	Dose			
	0	10	100	1000
Mammary Gland				
Adenoma only	1/90	0/80	1/80	2/80
Fibroadenoma only	21/90	18/80	10/80	19/80
Adenoma and/or fibroadenoma only	23/90	20/80	11/80	21/80
Carcinoma	16/90	13/80	20/80	40/80
Adenoma/Fibroadenoma/ Carcinoma	39/90	33/80	31/80	61/80
Pituitary				
Adenoma only	73/90	57/80	63/79	61/80
Carcinoma	1/90	3/80	0/79	6/80
Adenoma and /or Carcinoma	74/90	60/80	63/79	67/80
Kidney Tubules				
Adenomas	0/90	0/80	0/80	2/80

GRAPH 1. SIMAZINE, SPRAGUE-DAWLEY RAT Study, Kaplan-Meier Survival Curve for Female Mammary Gland Fcc.e Adenomas, Fibroadenomas, and/or Carcinomas