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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: June 8, 1981

SUBJECT: EPA Reg.#100-543, Technical Propazine; 6(a)(2) Data
CASWELL#184 Accession#243350-58

FROM: William Dykstra, Toxicologist
Toxicology Branch, HED (TS-769) WJD for LDC 6/10/81

TO: Robert Taylor (25)
Registration Division (TS-767) d.f. for WJB

Recommendations:

1. Technical propazine was not oncogenic in the 2-year mouse feeding study. The study is acceptable as Core-Minimum Data.

2. Technical propazine was considered weakly oncogenic to the mammary gland of female rats at 1000 ppm in diet. This finding triggers an oncogenic RPAR criterion. The study is acceptable as Core-Minimum Data.

3. The NOEL for reproductive parameters in the three-generation rat reproduction study was 100 ppm of technical propazine in the diet. The study is acceptable as Core-~~Minimum~~ GUIDELINE Data.

Review:

1. 2-Year Carcinogenicity Study in Mice (IRDC Report No. 382-004; April 24, 1980)

Test Material: Propazine technical; ARS No. 2046/76; Batch No. FL-76 1357; 35 lbs; white powder

Two hundred forty male (weighing from 21 to 28 grams) and 240 female (weighing from 20 to 25 grams) weanling Charles River CD-1 mice were initiated in this 2-year carcinogenicity study. The mice were housed individually in hanging wire-mesh cages and maintained in a temperature-, -humidity-, and light- (12-hr light/ 12-hr dark) controlled room. Water and the appropriate diets were available ad libitum throughout the study.

The mice were ear punched to identify treatment group. Beginning on December 17, 1976, ear punch verifications were recorded at each cage change.

The study was initiated on November 3, 1976. During the 5 weeks following initiation, three replacement mice were substituted for the following animals; a control female (#24827 replaced by #2503) that died (11/9/76), a mid-dose male (#24999 replaced by #25204) reported missing (11/9/76), and a mid-dose female (#25079 replaced by #25205) found dead (11/30/76). The rest of the replacement mice were appropriately sacrificed and discarded at the end of the 5-week period (December 8, 1976). The study was terminated on November 2 and 3, 1978.

In accordance with a computer-generated table of random numbers, the mice were selected and assigned to groups as follows:

Dose Level ppm	No. of Mice Initiated	
	Male	Female
0 (control)	60	60
3	60	60
1000	60	60
3000	60	60

The mice were observed three times daily (twice daily on weekends and holidays) for signs of overt toxicity, moribundity, and mortality. Detailed observations were recorded weekly as were the incidence, size and location of palpable masses.

Individual body weights were recorded monthly. Group mean food consumption was measured weekly. This was accomplished by weighing the food to be used for each group and then distributing it among the food jars in that group. At the end of the week, the food remaining in the jars was collected by groups and weighed. From this mean, individual food with compound and compound consumption values were calculated monthly.

At the completion of the experimental period, surviving mice from all groups were sacrificed by carbon dioxide asphyxiation and necropsied. At necropsy, an examination was made of the external body surfaces and orifices. Each mouse was then opened and contents of cranial, thoracic and abdominal cavities examined for any gross abnormalities. Tissues from each mouse, including the eviscerated carcass was collected for fixation in buffered 10% formalin.

Mice that died during the course of study were also necropsied and tissues collected as above.

Microscopic examination of formalin fixed, hematoxylin and eosin stained paraffin sections was performed for all mice in the control and high-dose groups. The following tissues were examined:

pituitary
peripheral nerve
thyroids/parathyroids
adrenal
trachea
esophagus
aorta
testes/ovaries
prostate/uterus
stomach
duodenum
small intestines (3 levels)
large intestines (2 levels)
urinary bladder
brain
spinal cord (3 levels)
eye and optic nerve
skeletal muscle
skin/mammary gland
lymph nodes (cervical mesenteric)
salivary gland
pancreas
liver
kidneys
spleen
heart
lung
sternum (bone marrow)
and any other tissues with lesions

Lymph nodes, thymus, spleen, and bone marrow were processed and examined in the mid- and low-dose female groups; additional sections were also prepared from tissues in these groups which were previously examined because gross lesions were noted at necropsy.

Statistical analyses of the data were performed.

Results:

No signs of overt toxicity were observed for any of the treated mice. Some incidental and intermittent signs seen in several control and treated mice were: corneal opacity, hair loss, tonic convulsions upon handling, soft stools, white internal eyes, extended and/or ulcerated penis, dilated pupils (unresponsive to light), tremors, functional and structural impairment of limbs, red material in vaginal opening, altered posture, labored breathing, and yellow material on ventral abdomen. A few palpable masses were observed in both control and treated mice, but the incidence was no greater for the treated animals than for the controls.

There were no compound-related effects observed on the rate of survival of the treated mice when compared with controls. Survival at week 104 was as follows:

Dosage Level ppm	No. Survivors/No. Initiated Male	Female
0 (control)	27/60	33/60
3	35/60	34/60
1000	37/60	27/60
3000	37/60	23/59*

*Mouse found missing, week 20.

Statistical analysis of the body weights through week 104 indicated that while there were occasional statistically significant values among the body weights of the treated mice when compared with controls, there were no compound-related effects observed with respect to body weight. Group mean body weights at week 104 were as follows:

Dosage Level ppm	Group mean body weight gms Male	Female
0 (control)	37	34
3	38	35
1000	37	35
3000	37	33

There were no compound-related effects apparent when the food consumption of treated mice was compared with that of the controls.

An increase in certain morphological changes were seen in the high-dose male and female mice in comparison to the control. In high-dose males, there was an increase above controls in focal myocardial fibrosis, centrilobular focal hepatocellular hypertrophy and focal glandular hyperplasia of the stomach. In high-dose females, there was an increase above controls in focal myocardial degeneration, focal sinusiodal lymphoid infiltrations of the liver, and diffuse hematopoiesis of the spleen. Amyloidosis was a degenerative lesion of common occurrence in almost all mice.

The prevalence was generally similar for control and treatment groups and the occurrence of amyloidosis was not considered compound-related.

Neoplasms were found with low prevalence in both control and treatment groups. The lung was the most common site of neoplasia with pulmonary (alveologenic) adenoma. The prevalence, however, of this spontaneous pulmonary neoplasm was not increased by compound administration. The initial evaluation showed an increase in the incidence of lymphoreticular cell tumors in females in the 3000 ppm group. Reevaluation of this data and examination of affected tissues in the 3 and 1000 ppm groups eliminated the apparent effect as shown in Table 1 below:

TABLE I

Incidence of Malignant Lymphoma/Reticulum cell Sacroma
*animal number

0		3 ppm		1000 ppm		3000 ppm	
Male	Female	Male	Female	Male	Female	Male	Female
24735*	24783	24858	24903	24971	25027	25108	25149
24756	24788	24863	24908	24982	25032	25119	25152
24767	24791	24876	24922	24986	25048	25139	25172
24772	24806	24881	24923		25056		25174
	24831		24942		25059		25177
	24842		24951		25062		25183
	25203		24952		25064		
			24960		25065		
					25072		
					25078		
4	7	4	8	3	10	3	6

Conclusion:

Technical propazine was not oncogenic in the 2-year mouse feeding study.

Classification: Core-Minimum Data

2. 2-Year Chronic Oral Toxicity Study in Rats with Technical Propazine (IRDC Report No. 382-007; April 28, 1980)

   Test Material: Propazine technical; ARS No. 2046/76; Batch No. FL-761357; 35 lbs; white powder

   Two hundred sixty male (weighing from 102 to 209 gm) and 260 female (weighing from 94 to 179 gm) weanling Charles River CD rats were selected randomly and initiated in this study.

   The rats were housed individually in hanging wire-mesh cages and maintained in a temperature-, humidity-, and light- (12-hr light/12-hr dark) controlled room. Test and control diets as well as water were available ad libitum throughout the study.

The basal laboratory diet was ground Purina Laboratory Chow. The rats were identified individually with numbered ear tags. Beginning on July 26, 1977, ear tag verifications were recorded at each cage change, before and after blood and urine sample collection and before necropsy. The study was initiated on July 27, 1976; there were two interim sacrifices, one at 12 months and a second at 13 months of study because of the following experimental procedure.

Ten additional male and 10 additional female rats were initiated in the control and high-dose groups; of these additional animals, five of each sex were sacrificed and necropsied after 12 months of study. The remaining five of each sex were placed into a compound-withdrawal group and fed a control diet for 4 weeks and then sacrificed and necropsied. During 4 week of study, Group III female 38160 replaced 39644 which died. The study was terminated on July 26-28, 1978.

Propazine technical was fed in the diet at the following dosage levels:

Dosage Level ppm	Number of Rats	
	Male	Female
0 (control)	70	70
3	60	60
100	60	60
1000	70	70

The rats were observed twice daily for signs of overt toxicity, moribundity and mortality. Detailed observations were recorded weekly.

Individual body weights were recorded weekly for the first 3 months and monthly thereafter. After one year of study individual body weights and food consumption were recorded weekly for rats placed on withdrawal.

Individual food with compound consumption values (for 10 rats/sex/group) were recorded weekly for the first 3 months and monthly thereafter. After one year of study, individual food consumption values were recorded weekly for the rats placed on withdrawal. Food efficiency was calculated through 30 weeks of study.

Blood and urine samples were obtained from 10 rats/sex for both the control and high-dose groups at 3, 6, 12, 18 and 24 months of study. Prior to sample collection the rats were housed overnight in metabolism cages (without food or water). The blood was obtained by the orbital sinus technique.

Hematologic tests included hemoglobin, hematocrit, total and differential WBC, total RBC, total platelet count, prothrombin time, and partial thromboplastin time.

Biochemical tests included fasting blood glucose, BUN, SGOT, SGPT, SAP, serum total protein and total cholesterol.

Urinalyses included a description of appearance, measurement of volume.

Five male and five female rats from the control group and the 1000 ppm group were sacrificed with carbon dioxide asphyxiation and necropsied after 12 months of compound feeding.

The five male and five female rats from these groups which were placed in compound withdrawal were sacrificed and necropsied after 4 weeks of compound withdrawal. All remaining rats were sacrificed and necropsied after 2 years of compound withdrawal. At necropsy, an examination was made of the external body surface and body orifices. The rat was then opened and the contents of the body cavities were examined in situ, removed and again examined. Liver, kidneys, spleen, heart and testes were weighed fresh at necropsy.

Representative tissues and organs from each rat were collected and fixed in phosphate buffered neutral 10% formalin. Adrenal glands, thyroid and ovaries were weighed after fixation.

Rats which died or were sacrificed in extremis during the course of the study were necropsied as above except no organs were weighed.

Hematoxylin and eosin stained paraffin sections were prepared at IRDC by standard histologic methods and examined microscopically from all rats from the control and 1000 ppm groups which were sacrificed after 12 months of study or which died or were sacrificed in extremis during the first 12 months of study.

adrenal gland
aorta
bone marrow
brain (cerebrum, cerebellum, pons)
cecum
colonm
esophagus
eye
gonads
harderian gland
peripheral nerve (sciatic)
pituitary gland
prostate
salivary gland (submaxillary)
skin
small intestines (duodenum, jejunum, ileum)
spinal cord
heart
kidney
liver
lung
lymph node (cervical and mes.)
mammary gland
muscle
optic nerve
pancreas
parathyoid
spleen
sternum
stomach (cardia, fundus, pylorus)
thyroid
trachea
urinary bladder
uterus
any other tissue with gross lesions

The above tissues from rats which were sacrificed at termination or which died or which were sacrificed in extremis during the period 12-24 months were delivered to Experimental Pathology Laboratories, Inc., Herndon, Virginia for histologic processing and microscopic examination.

Statistical analyses of the data were performed.

Results:

No signs of overt toxicity were observed among treated animals. Incidental findings seen occasionally among control and treated rats included skin lesions, hair loss, material around eyes and nose, material around anogenital region, lacrimation, corneal opacity, labored breathing and respiratory congestion, discolored urine, soft stools, swollen hind feet (hard to the touch in several cases), raised pink areas on ventral surfaces, exposed areas of skin and eyes pale, excessive salivation and ventral neck swollen.

Palpable masses were observed and recorded in all groups; there were no greater numbers of masses in treated rats than in controls. The number of palpable masses in the rats at 104 weeks of the study were as follows:

	I (0)		II (3 ppm)		III (100 ppm)		IV (1000 ppm)	
	Male	Female	Male	Female	Male	Female	Male	Female
No. of masses	9	57	19	75	18	75	15	82
Survival (104 wks)	31/60	36/60	42/60	37/60	46/60	46/60	38/60	25/60
% rats with masses	19	75	31	73	30	72	32	92
% with single masses	67	37	69	37	71	36	92	30
%with multiple masses	33	63	31	63	29	64	8	70

All groups generally showed a decrease in rate of body weight gain with an increase in dosage of compound. A t-test comparison between means and the ratio of change in body weights of control and treated groups showed that for female rats of the low-dose (3 ppm), a statistically significant decrease occurred between weeks 26 through 65; for female rats of the middle-dose (100 ppm), a statistically significant decrease occurred between weeks 0 through 104; for female rats of the high-dose (1000 ppm), a statistically significant decrease occurred between weeks 0 through 104. Similarly, for male rats at the low-dose (3 ppm), a statistically significant decrease occurred between weeks 0 through 104; for male rats at the middle-dose (100 ppm), a decrease (though not statistically significant) occurred though most of the weeks from 0 to 104; and for high-dose (1000 ppm) male rats, a statistically significant decrease occurred through weeks 0 to 104.

There was little difference in the amount of food consumed per day between the control and treated rats, although a slight decrease was noted for both males and females in the high-dose group. This slight decrease in food consumption was not enough to account for the significant decrease in body weights. There were no compound-related effects in hematologic tests, biochemical tests and urinalyses; although a few values were statistically significant, all were within the expected ranges, and no trends of increase or decrease were evident.

Although statistical variations occurred in sex group mean weights of a number of organs of rats in the treated groups, there was no dose response evident and the organs which had statistical weight variations were not the site of compound-related gross or microscopic morphologic lesions. These weight variations therefore were not considered of toxicological significance.

The number of subcutaneous masses and nodules in female rats from the 1000 ppm group was slightly increased when compared to the control group at gross necropsy.

12-Month Interim Sacrifices, Deaths 0-12 Months

No microscopic pathologic lesions which were considered related to Propazine feeding were seen in any tissues examined from rats from the 1000 ppm group which were sacrificed at the 12-month interim or which died or were sacrificed *in extremis* during the first 12 months of study. Microscopic findings in these rats were those which commonly occur in untreated rats of this age and strain. They were primarily lesions of mild inflammatory conditions or early degenerative changes and they occurred with similar frequency and severity in rats from the control group and 1000 ppm group.

Terminal Sacrifices, Deaths 12-Months to Termination

A variety of microscopic changes was observed in most of the organs and tissues in both the control and high-dose (1000 ppm) groups of rats. These occurred either infrequently or with similar distribution between the two groups and are considered unrelated to the exposure to the compound. These changes were most evident in the lungs and kidneys. The lung changes were representative of the chronic respiratory disease complex (Murine Respiratory Mycoplasmosis). These changes included varying degrees of mutifocal to diffuse pneumonitis with peribronchial and perivascular lymphnoid cell accumulations and focal accumulations of foamy macrophages. Multifocal hemorrhages of the lung were also observed in both the control and treated rats changes related to this complex were also observed in the tracheas and hearts of individual rats in both the control and treated groups.

The changes in the kidneys were compatible with the microscopic lesions of the chronic progressive glomerulonephrosis and nephritis observed in most rat strains.

Incidental changes were present in the livers from both control and treated rats. A low incidence of hepatocellular carcinoma and adenoma occurred in both the control and treated rats. Other hepatic changes, seen with a higher frequency but with similar distribution in the control and treated rats, included bile duct hyperplasia, multifocal hepatocytomegaly and multifocal to diffuse vacuolation.

A generally low incidence of neoplasms was observed in most organs and tissues from both the control and treated groups except for the pituitary, testes and mammary gland. Pituitary adenomas and carcinomas were commonly observed, although evenly distributed among the control and treated groups, there was a higher incidence in the female rats. Interstitial cell tumors occurred in the testes of some rats in the control and treated groups. There was a slightly higher incidence of this tumor in the high-dose group of rats (8/64, control vs. 12/64, high-dose) which was considered to be a biological variation and not a treated-related change.

Three was a high incidence of hyperplastic mammary gland changes in the control and all three test groups of female rats. The severity of these hyperplasia changes of the mammary glands made it difficult in individual rats with mammary gland neoplasms to classify the type of mammary gland tumor present in the animal. Areas of glandular hyperplasia (lobular) were present in areas of relatively normal mammary gland as well as within the benign adenomas and fibroadenomas observed in both the control and all three groups of treated animals. The classification of mammary gland tumors used in the report is set forth in the Pathology of Tumors in Laboratory Animals, Volume I, Tumours of the Rats, Part I by V.S. Turusov. Individual animals, the histological differentiation between adenomas and well differentiated carcinomas of the mammary gland was made difficult due to the degree of hyperplastic change present. Classification of the tumors as adenocarcinomas or papillary carcinomas was used when one or more of the following criteria was present within the tumor: (1) loss of normal glandular architecture; (2) pronounced variability in cytologic features; (3) prominent nucleoli; (4) numerous mitotic figures; (5) multiple layering of the epithelium; (6) lack of cellular orientation; and (7) local invasion.

The male rats in this study had very few tumors of the mammary gland. No tumors of the mammary glands were present in the male or female rats which died before the twelve-month sacrifice. The female control rats and rats exposed for a longer period of time had a high incidence of mammary gland tumors. The distribution of the mammary gland tumors in the female rats from the two-year sacrifice is presented in Table I.

TABLE I
Distribution of Mammary Gland Tumors

	Group I Control	Group II 3 ppm	Group III 100 ppm	Group IV 1000 ppm
No. Examined	(55)	(57)	(60)	(55)
TYPE OF TUMORS				
Adenomas No./Rat	3/3	3/3	5/5	10/10
Fibroadenoma No./Rat	34/22	37/22	31/24	35/24
Adenocarcinoma No./Rat	9/6	12/11	11/8	13/9
Papillary Carcinoma No./Rat	4/4	12/7	4/3	12/8
MALIGNANT TUMORS				
Total/Rat	13/9	24/17	15/10	25/14*
Average Tumor/Rat	1.44	1.41	1.50	1.78
Percentage of Tumor-Bearing Rats	16.4%	29.8%	16.7%	25.5%
BENIGN TUMORS				
Total/Rat	37/24	40/25	36/26	45/30**
Average Tumor/Rat	1.54	1.60	1.38	1.50
Percentage of Tumor-Bearing Rats	43.6%	43.9%	43.3%	54.5%
TOTAL MAMMARY GLAND TUMORS				
Total/Rat	50/28	64/33	51/32	70/40***
Average Tumor/Rat	1.78	1.94	1.69	1.75
Percentage of Tumor-Bearing Rats	50.9%	57.9%	53.3%	72.7%

*Twelve tumors in two rats.
**Nine tumors in two rats.
***Twenty-one tumors in four rats.

The most frequent mammary gland tumor present in female rats was the fibroadenoma which had a fairly equal distribution among the control and the three treatment groups.

Adenomas had a similar distribution in the control and treatement Groups II (3 ppm) and III (100 ppm) while there was an increase in the number of adenomas in Group IV (1000 ppm). Adenocarcinomas were fairly equally distributed among the control and three treatment groups.

Papillary carcinomas were similar in occurrence in the control group and Group III (100 ppm) with an increased incidence in Group II (3 ppm) and Group IV (1000 ppm).

A comparison of the number of female rats having mammary gland tumors shows a higher incidence of animals with mammary gland tumors in the high dose group (1000 ppm) when compared to the control group. The increase is due to an increase in the incidence in both benign and malignant tumor-bearing animals in the high-dose group.

A difference in the number of tumor-bearing animals is not observed between the low (3 ppm), middle (100 ppm) and control group of female rats in this study. A comparison of the number of tumors per individual animal does not show any substantial difference between these ratios in the control and the three treated groups of rats with respect to the total number of mammary gland tumors, malignant tumors or benign tumors. However, evaluation of the individual animals in the high dose group (1000 ppm) shows two animals (No. 39814 and No. 39832) with twelve malignant mammary gland tumors (seven and five tumors, respectively) and two animals (No. 39799 and No. 39804) with nine benign tumors (five and four, respectively). This is a total of twenty-one tumors in four rats from the high dose group. Even though there is an apparent increase incidence of mammary gland tumors in the high dose group, it may be difficult to conclude that this increased incidence of mammary gland tumors was related to the exposure to 1000 ppm of Propazine technical. A high incidence of mammary gland tumors occurred in all groups of female rats in this study. Instances of fifty-five percent, sixty-two percent, sixty-four percent and as high as eighty-five percent have been reported in Sprague-Dawley rats (Sher, Sanford P., Toxicology and Applied Pharmacology, 22 (1972); pp. 562-588.) These data, however, did not come from IRDC.

Conclusions:

A statistically significant (Fisher's Exact test, P = 0.015) increase in rats bearing mammary tumors occurred in the high-dose female rats. The increase in the total number of tumor-bearing animals in the high-dose group may reflect a biological variation in Sprague-Dawley rats. The historical control data of mammary tumors in Charles River CD rats at IRDC from 1975-1979 has been submitted by the registrant and the number of female rats bearing mammary tumors compared to the number of female rats examined is 769/1528 (50.3%). The historical control data was compared to the high-dose female rats using 2x2 contingency Chi-Square analysis. A statistically significant increase (p = .0011) was seen in the mammary adenomas and number of tumoring bearing rats in the T-III females (1000 ppm).

Therefore it can be concluded that Propazine technical at dietary levels of 1000 ppm was weakly oncogenic to female rats producing increased mammary gland tumors.

(21)

Test for Significance of Differences Between Proportions 11/13/80

Mammary neoplasms in rats

ppm	# RESP	Total	%	+/-2(S.D.)	One Tail P Statistic Fisher's
0.000	28	55	50.91	+/-(14.12)	
3.000	33	57	57.89	+/-(13.69)	0.290
100.000	32	60	53.33	+/-(13.46)	0.471
1000.000	40	55	72.73	+/-(12.68)	0.015

Test for Linear Trend in Proportions P = 0.015

The pathologist for the study had difficulties in attributing these findings to administration of propazine for the following reasons:

1) Most of the mammary glands in the rats in this study (control and treated) had some degree of hyperplastic change probably due to some extent to the large number of pituitary tumors in the rats in this study.

2) A high incidence of mammary gland tumors occurred in all groups of female rats in this study. Instances of fifty-five percent, sixty-two percent, sixty-four percent and as high as eighty-five percent have been reported in Sprague-Dawley rats (Sher, Sanford P: 1972, Tox. Appl. Pharmacol. 22: 562-588.

3) There was an absence of any significant increase in the number of tumors in the mammary glands of male rats or in any of the rats sacrificed prior to twelve months on study.

4) A similar distribution and incidence of mammary gland tumors in the control group and in Group III (100 ppm) demonstrated a lack of dosage-related response.

Classification: Core-Minimum Data

3. Three-Generation Reproduction Study in Rats with Propazine Technical (IRDC Report No. 382-010; August 10, 1979)

Test Material: Propazine technical; ARS No. 2046/76; Batch No. FL-761357; 35 lbs.; white powder

Forty male (weighing from 121 to 175 gm) and 80 female (weighing from 96 to 148 gm) Charles River CD rats were initiated on this study. The rats were evenly distributed among each of three treatment groups and one control group (10 males/group and 20 females/group). Placement of the rats was made so initial group mean body weights for each sex were similar. Littermates, by sex, were evenly distributed among the groups.

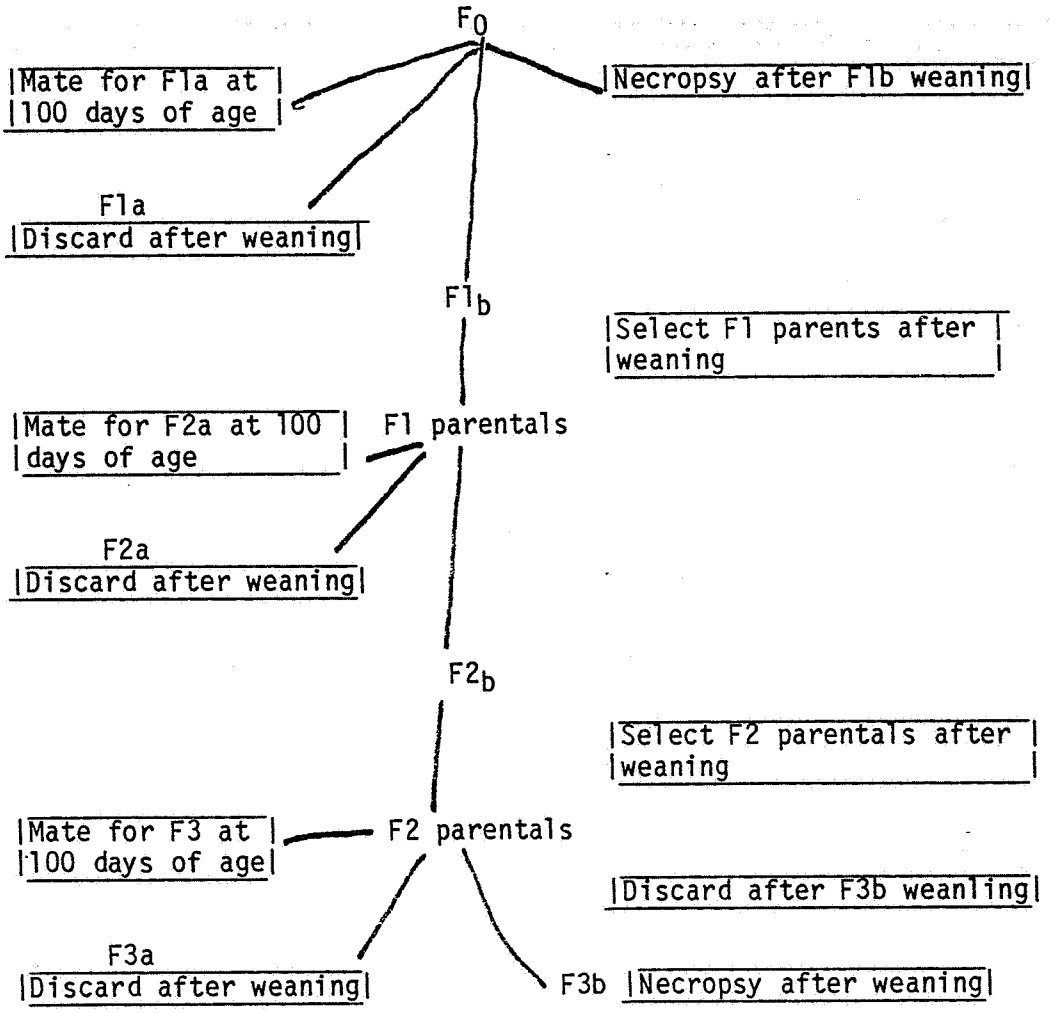
Except during mating and through lactation, the rats were individually housed in hanging wire-mesh cages. During the initial mating periods, the rats were housed in units of one male and two females in plastic boxes on ground corn-cob bedding.

During the second or third remating periods, some males were housed with one female. Following the mating periods and during lactation, the females were individually housed in plastic boxes on ground corn-cob bedding and the males in hanging wire-mesh cages. Throughout the span of this study, the rats were housed in a temperature-, humidity-, and light- (12 hours on/off) controlled room. Tap water and the control and test diets were available ad libitum.

The study was initiated on September 7, 1976 and terminated with the last sacrifice on July 3, 1978.

Propazine technical was administered in the diet at fixed percentages to achieve dosage levels of 3, 100 and 1000 ppm . Ten male and 20 female rats were initiated at each treatment level and one control group.

Shown below is the breeding schematic used in the study.



The control and treated rats were maintained on their respective diets throughout the duration of the first generation (F_0). After 77 days of treatment and at approximately one hundred days of age, the F_0 parental rats were initially housed in units of one male and two females within the same treatment group to produce the $F1_a$ litters. The rats were housed together for a maximum of 21 days. The females were vaginally smeared daily during this period until sperm or a copulatory plug was observed. This finding was designated gestation day 0.

If no evidence of mating was observed after two estrous cycles (appproximately ten days), those females were housed with a different male within the same treatment group for an additional 10 days. This procedure was repeated once. No more than three different males were used with each female during the mating period. Just prior to expected parturition or at the end of the maximum mating period, the rats were separated and individually housed. The females were allowed to deliver. The day all pups in a litter were found was designated lactation day 0. During lactation, the pups were counted, sexed and weighed at designated intervals. At weaning, the Fla pups were examined for external abnormalities, sacrificed and discarded.

After weaning, the F_0 parental females were allowed a minimum 10-day rest period and then mated a second time to produce the F_{1b} litters. The mating procedure was identical to the F_{1a} mating, except the females were housed with different males within the same treatment group.

The rats were housed together for a maximum of 30 days. The females were vaginally smeared daily during this period until sperm or a copulatory plug was observed (gestation day 0).

If no evidence of mating was observed after two estrous cycles, those females were housed with a different male within the same group for an additional 10 days. This procedure was repeated once. No more than three different males were used with each female during the mating period.

Just prior to expected parturition or at the conclusion of the maximum mating period, the rats were separated and individually housed. The females were allowed to deliver.

The day all pups in a litter were found was designated lactation day 0. The F_{1b} pups were counted, sexes and weighed on designated days during lactation. After weaning, 10 male and 20 female F_{1b} pups were selected from each group to comprise the second generation (F_1) parents. Also after weaning and following approximately 33 weeks on test, all surviving male from each group were sacrificed and necropsied. Any F_{1b} pups not selected for study continuation and the remaining parental females were sacrificed and discarded.

The F_1 parental rats, selected from the F_{1b} litters, remained on their respective control or treated diets during the span of this generation. At approximately 100 days of age, the parental rats were mated to produce the F_{2a} litters. The mating procedure was identical to the F_{1a} mating with avoidance of brother-sister matings. The rats were housed together for a maximum of 30 days. Parental and pup observations conducted during the gestation and lactation periods were identical to those employed for the F_{1a}. At weaning, the F_{2a} pups were examined for external abnormalities, sacrificed and discarded.

Following a minimum of 10 days after weaning, the F_1 parental rats were mated a second time in a manner identical to the F_{1b} mating and avoiding brother-sister matings to produce the F_{2b} litters. The rats were housed together for a maximum of 31 days. Parental and pup observations conducted during the gestation and lactation periods were identical to those employed for the F_{1b}.

After weaning, 10 male and 20 female F_{2b} pups were selected from each group to comprise the third generation (F_2) parents. Also after weaning, all surviving male and 10 female F_1 parental rats from each group were sacrificed and necropsied. Any F_{2b} pups not selected for study continuation and the remaining parental females were sacrificed and discarded.

The F_2 parental rats, selected from the F_{2b} litters, remained on their respective control or treated diets until termination of the study.

At approximately 100 days of age, the parental rats were mated to produce the F_{3a} litters. The mating procedure was identical to the F_{1a} mating with avoidance of brother-sister matings. The rats were housed together for a maximum of 31 days. Parental and pup observations conducted during the gestation and lactation periods were identical to those employed for the F_{1a}. At weaning, the F_{3a} pups were examined for external abnormalities, sacrificed and discarded. Following a minimum of 10 days after weaning, the F_2 parental rats were mated a second time in a manner identical to the F_{1b} mating and avoiding brother-sister matings to produce the F_{3b} litters. The rats were housed together for a maximum of 30 days. Parental and pup observations conducted during the gestation and lactation periods were identical to those employed for the F_{1b}.

After weaning, 10 male and 10 female F_{3b} pups were selected from each group, sacrificed and necropsied. Also after weaning, all surviving males and 10 female F_2 parental rats from each group were sacrificed and necropsied. The remaining parental females and F_{3b} pups were sacrificed and discarded.

The parental rats and pups were observed daily for signs of overt toxicity, changes in general behavior and appearance and mortality. Detailed observations, individual body weights and food consumption were recorded on a weekly basis for the parental rats. Specific observations for the reproduction aspects of the study included male and female fertility, length of the gestation period, numers of male and female pups at weaning and the viability, growth and survival of the pups through weaning. The number of pups surviving at lactation days 0, 5, 14 and 21 were recorded. Litter size was reduced to 10 pups of equal sex ratio, if possible, on day 5 of lactation. Individual pup body weights were recorded on day 21 of lactation.

As mentioned previously, at intervals during the study, 10 male and 10 female F_0, F_1 and F_2 parental rats from each group were sacrificed with carbon dioxide and necropsied. Ten male and 10 female rats from each group from the F_{3b} generation were also sacrificed at the conclusion of the study. At necropsy, contents of the cranium, thorax and abdomen were examined in situ and after removal. Representative tissues from each rat were collected and fixed with buffered 10% neutral formalin. All pups and parental rats which died during the course of study were also necropsied. Hematoxylin and eosin stained paraffin sections of the following tissues were prepared and examined from rats in the control and high dose group of the F_0, F_1 and F_2 parental rats and the F_{3b} weanling rats:

adrenal gland
bone marrow (sterum and femur)
brain (cerebrum, cerebellum, pons)
large intestine (2 levels)
esophages
eye
heart
kidney
liver
lymph node (cervical and mesenteric)
mammary gland
optic nerve
ovary
pancreas
parathyroid
peripheral nerve (sciatic)
pituitary gland
prostate
salivary gland (parotid, sublingual, submaxillary)
seminal vesicles
small intestines (3 levels)
spinal cord (3 levels)
spleen
stomach
testes
thyroid
trachea
urinary bladder
uterus
lung

Organs underlined above were weighed at necropsy.

Statistical analyses of the data were performed.

Results:

No changes considered to be related to treatment of Propazine technical were seen in parental rats in relation to the general behavior, appearance or survival of the treated rats when compared to the controls. No difference were seen between the pups in the control and treated groups with respect to the general behavior, appearance or survival which was considered treatment related. Differences in the mean body weights of the parental rats receiving Propazine technical at dosage levels of 3 and 100 were not considered treatment related. At study week 63, mean body weights of the F_2 100 ppm parental females were statistically significant higher than the control females. This difference was not considered treatment related. At the 1000 ppm treatment level, the parental mean body weights of both the males and females were generally lower than the control group throughout treatment of Propazine technical. Statistical significance of these differences were not evident at all points of analysis. At this treatment level, the mean body weights of the F_0 females at study weeks 10 and 33; the F_1 males at study week 63, the F_1 females at study weeks 41 and 63 and the F_2 females at study weeks 72 and 95 were statistically significantly lower than their respective control group.

No treatment-related differences were seen between the control and treated groups with respect to male and female fertility, the length of the gestation periods and the viability and survival of the pups through weaning. The gestation survival index of the F_{2b} litters in the 100 ppm treatment group was statistically significant higher than the control group, but this difference was not considered a result of treatment. No biologically meaningful or statistical significant differences were seen in the mean pup body weights of the litters at lactation day 21 in the 3 and 100 ppm treatment groups when compared to the control itters. The mean pup body weights of each of the six litters producted (F_{1a}, F_{1b}, F_{2a}, F_{2b}, F_{3a}, F_{3b}) at the 1000 ppm treatment level were consistently lower than the mean weights of the control pups. Statistical significance was noted in all but the F_{1a} litters at this treatment level.

No gross pathological lesions or abnormalities which are considered compound-related were seen at necropsy in any F_0, F_1, F_2 parental rats or F_{3b} weanling rats which were sacrificed at termination or which died during the course of study and were examined.

Statistiical analysis or organ weights showed the following significantly different means in the treatment groups when compared with the control groups. In the absence of any morphologic change, the biological significance of these organ weight variations is unknown.

Organ	Dosage Level ppm	Sex	Weight	Change	p <
F_0 Generation					
testes	1000	M	relative	increase	0.05
heart	1000	M	relative	increase	0.05
F_1 Generation					
liver	1000	M	relative	increase	0.05
heart	1000	M	relative	increase	0.05
F_2 Generation					
liver	3	F	relative	decrease	0.05
	1000	M	absolute, relative	decrease	0.01, 0.05
	1000	F	absolute	decrease	0.05
kidneys	1000	M	absolute	decrease	0.05
testes	1000	M	relative	increase	0.01
ovaries	100	F	absolute, relative	decrease	0.01, 0.01

No microscopic morphological changes considered compound-related were seen in the F_0, F_1, F_2 parental rats or F_{3b} weanling rats sacrificed at termination.

Conclusion:

The NOEL for reproductive parameters in the study is 100 ppm. The LEL is 1000 ppm and the reproductive effect was statistically significantly reduced mean pup body weights in five of six litters.

Classification: Core-Guideline Data

TS-769:th:TOX/HED:WDykstra:6-8-81:#1