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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

MAY - 8 1997

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Propazine (3rd)

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and
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TO: Robert Taylor/Terri Stowe
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and
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Special Review and Reregistration Division (7508W)

The Health Effects Division (HED) Carcinogenicity Peer Review Committee (CPRC) met on August 28, 1996, to discuss and evaluate the weight-of-the-evidence on Propazine with particular reference to its carcinogenic potential. It should be noted that the evaluation of Propazine will be revisited in the near future, as part of a consideration of the Triazines as a class. At the present meeting, the CPRC concluded that Propazine should be classified as Group C - possible human carcinogen - and recommended that for the purpose of risk characterization a low-dose extrapolation methodology (Q_1^*) be applied to the animal data. This was based on statistically significant increases in mammary gland adenomas, carcinomas and combined adenomas/carcinomas in female Sprague-Dawley rats, genotoxicity, and SAR to other triazine pesticides. The CPRC recommended that the quantification of human risk (Q_1^*) be based on the combined mammary gland adenomas and carcinomas in the female rat.

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SUMMARY

Propazine has been previously evaluated twice by the HED Carcinogenicity Peer Review Committee (CPRC). The CPRC first classified propazine as a Group C carcinogen with a Q_1^* based on the statistically significant increase in malignant mammary gland tumors in female Sprague-Dawley rats at the 1000 ppm dose level (highest dose tested) [Peer Review of Propazine, Aug. 10, 1987].

The second CPRC, based on re-evaluations (by the same pathologist) of 3 control and 12 high dose slides (in which most of the carcinomas in the treated group were rediagnosed as adenomas) classified propazine as a Group C carcinogen without quantification by a linear model [Peer Review of Propazine - Re-Evaluation, Jan. 10, 1989.

> It was noted at the second CPRC that the registration of propazine had been withdrawn by Ciba-Geigy and it was stated that in light of the discrepancies in the tumor counts, a complete independent re-reading of all the slides would be required, if the reregistration of propazine was ever to be reconsidered.

To comply with the requirements of the second peer review, a new potential Registrant of propazine completed an independent re-review of all mammary gland slides of female rats in the 2-year rat feeding study. In order to resolve differences in diagnosis between the original study pathologist and the reviewing pathologist, a Pathology Working Group (PWG) Peer Review was conducted, in accordance with Pesticide Regulation Notice 95-4.

Based on the results of the PWG review, administration of Propazine in the diet to Sprague-Dawley rats resulted in a statistically significant increase in mammary gland adenomas, carcinomas and combined adenoma/carcinoma at the highest dose (1000 ppm); there was a statistically significant increase for carcinomas at the lowest dose (3ppm) as well. There were also statistically significant positive trends for adenomas, carcinomas and combined adenoma/carcinoma. There were no statistically significant increases in tumors in male rats.

The incidences of the adenomas and carcinomas in female rats at the highest dose exceeded the means of the historical control data.

The CPRC agreed that the highest dose in both sexes was adequate, and not excessive, based on body weight gain depressions of 10 - 15%. There was no other evidence of toxicity (a non-statistically significant increase in mortality in female rats was not considered

to indicate excessive toxicity.)

The mouse study which had been evaluated at the previous Peer Review(s) (negative at adequate dose) was not revisited by the CPRC.

Propazine was positive in a gene mutation assay with V79 Chinese hamster cells without microsomal activation, and to a lesser extent with activation but was negative in a nucleus anomaly assay and a DNA damage and repair assay.

Propazine is structurally related to other triazines (Cyanazine, Simazine, Atrazine, Terbutryn) which also produced mammary gland tumors in rats and were negative in the mouse and had mutagenic activity (Atrazine was negative in three submitted acceptable assays, although there are some positive results in the published literature).

The classification of Group C with a (Q_1^*) for quantification of human risk was based on the increases in mammary tumors, with a malignant component, positive results in a gene mutation assay and strong SAR to other triazines.

A. Individuals in Attendance at the meetings:

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

Stephanie Irene

William Burnam

Karl Baetcke

Kerry Dearfield

Esther Rinde

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

William Dykstra[1]

Lori Brunsman

Lucas Brennecke[2] (PAI/ORNL) SEE 4a

3. Other Attendees:

Kit Farwell, Bernice Fisher, Albin Kocialski, Ed Budd, Kathryn Boyle (HED).

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

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B. Material Reviewed

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Dykstra, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

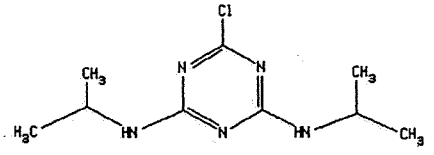
Propazine is 2-chloro-4,6-bis(isopropylamino)-s-triazine. It is a herbicide and controls annual grassy weeds for sorghum. Tolerances are established on sweet sorghum, fodder, forage, and grain at 0.25 ppm in 40 CFR 180.243.

Propazine has been to the HED Carcinogenicity Peer Review Committee (CPRC) twice before. On August 8, 1987, the CPRC first classified propazine as a Group C carcinogen with a Q_1^* of 0.17 $(mg/kg/day)^{-1}$ based on the statistically significant increase in malignant mammary gland tumors in female Sprague-Dawley rats at the 1000 ppm dose level (highest dose tested).

The second CPRC, based on re-evaluations (by the same pathologist) of 3 control and 12 high dose slides in which most the carcinomas in the treated group were rediagnosed as adenomas, classified propazine as a Group C carcinogen without quantification. It was noted that the registration of propazine had been withdrawn by Ciba-Geigy. In light of the discrepancies in the tumor counts, a complete independent re-reading of all the slides would be required, if the reregistration of propazine was ever to be reconsidered.

To comply with the second peer review, the Griffin Corporation, a new potential Registrant of propazine, completed an independent re-review of all mammary gland slides of female rats in the 2-year rat feeding study, with a draft report dated August 12, 1994. Griffin Corporation then requested a Pathology Working Group (PWG) Peer Review of the proliferative lesions of the mammary glands of female rats be conducted by Experimental Pathology Laboratories, Inc. (EPL) to resolve differences in diagnosis between the original study pathologist and the reviewing pathologist. The PWG was conducted November 30, 1994, and the final report dated January 20, 1995. The results of the PWG are presented in this memo.

STRUCTURE of PROPAZINE



Propazine

D. Evaluation of Carcinogenicity Data

1. Chronic Toxicity/Carcinogenicity Study in Rats

Reference: D. Clifford Jessup; Two Year Oral Chronic Toxicity Study in Rats (April 18, 1981); IRDC #382-007; MRID NO.: 00041408 ,

a. Experimental Design

Randomized groups of 60/sex/dose Sprague-Dawley rats were fed dietary levels of 0, 3, 100, or 1000 ppm (**0, 0.15, 5.0, or 50 mg/kg/day**) for 2 years. An additional 10/sex were added to the control and high dose groups for interim sacrifice at 12 months (5/sex) and a 4 week "recovery period" for 5/sex control and high dose animals. Hematology, clinical chemistry and urinalyses were conducted on 10/sex from control and high dose groups at 3, 6, 12, 18, and 24 months. All animals were necropsied, organ weights were taken at 12 and 24 months and 65/sex from control and high dose were examined microscopically. Mammary gland tissue from all male and female rats in all dose levels was examined microscopically.

b. Discussion of Tumor Data

Mammary gland tumors (adenocarcinomas) were significantly increased above controls in 3 and 1000 ppm females and adenomas were increased above controls at 1000 ppm. These mammary gland tumors were considered compound related. Other tumor types were comparable between control and treated high dose rats of both sexes.

Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls, for mammary gland adenomas, and adenomas and/or adenocarcinomas combined, all at $p < 0.01$. There was also a

significant increasing trend, and significant differences in the pair-wise comparisons of the 3 and 1000 ppm dose groups with the controls, for mammary gland adenocarcinomas, all at $p < 0.05$. In addition, there was a significant difference in the pair-wise comparison of the 3 ppm dose group with the controls for mammary gland adenomas and/or adenocarcinomas combined at $p < 0.01$. There were no statistically significant increases in mammary gland fibroadenomas.

The statistical analyses of the female rats were based upon Peto's Prevalence Test since there was a statistically significant positive trend for mortality with increasing doses of Propazine in female rats. See Table 1 for tumor analysis results.

Table 1. Propazine - Sprague-Dawley Rat Study

Female Mammary Gland Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	3	100	1000
Adenomas (%)	1/52 (2)	4/55 (7)	4/58 (7)	9[a]/52 (17)
p =	0.001**	0.127	0.124	0.004**
Adeno-carcinomas (%)	5/57 (9)	13[b]/58 (22)	8/59 (14)	13/55 (24)
p =	0.047*	0.025*	0.222	0.014*
Combined (%)	6/57 (11)	17/58 (29)	11[c]/59 (19)	21[c]/55 (38)
p =	0.001**	0.009**	0.116	0.0002**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 77, dose 1000 ppm.

[b]First adenocarcinoma observed at week 50, dose 3 ppm.

[c]One animal in each of the 100 and 1000 ppm dose groups had both an adenoma and an adenocarcinoma.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

IRDC Historical Controls (1976-1979)

Microscopic Diagnosis	Mean	Range
Adenoma	9.5%	2% - 22%
Fibroadenoma	38.6%	19% - 60%
Adenocarcinoma	11.8%	1% - 29%
Mammary Tumors Combined	50.3%	33% - 68%

The registrant submitted the incidences of mammary gland tumors in 22 historical control studies with Sprague-Dawley rats from IRDC studies conducted between 1975-1979.

The propazine study was conducted between 1976 -1978. The incidence of adenocarcinomas in the 1000 ppm propazine treated group (24%) exceeded the 20 of the 22 historical control studies and at 3 ppm, the incidence of adenocarcinomas in the propazine treated group (22%) exceeded 19 out of 22 historical control studies. With respect to adenomas, the incidence in the 1000 propazine treated group (17%) exceeded 16 out of 18 historical control studies.

c. Nonneoplastic Lesions: There were no nonneoplastic lesions associated with the mammary gland tumors in the propazine rat study.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The decreased body weight and weight gain in both sexes (♂, 10-13% decrease in BW and 16% decrease in BW gain; ♀, 7-11% decrease in body weight and 16-27% decrease in weight gain)) at the high dose is considered toxicologically significant and evidence that adequate high dose levels in both sexes were used to assess carcinogenicity.

The NOEL is 100 ppm (5 mg/kg/day). The LEL is 1000 ppm (50 mg/kg/day) and the effect is decreased body weight.

The statistical evaluation of mortality indicated a significant increasing trend with increasing doses of Propazine in female rats. The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program. However, since at 1000 ppm (HDT),

most of the increased mortalities occurred between weeks 90-105. However, the increase in deceased female rats at 1000 ppm prior to completion of the study were not considered related to propazine, since the additional mortalities were not associated with mammary gland tumors or toxicity, other than decreased weight gain.

2. Reference: Jessup, D.C. (1980); 2-Year Carcinogenicity Study in Mice; IRDC # 382-004; April 24, 1980; MRID No. 00044335

a. Experimental Design

Randomized groups of 60/sex/dose CD-1 mice were fed in the diet at doses of 0, 3, 1000, or 3000 ppm (**0, 0.45, 150, or 450 mg/kg/day**) for two years with technical propazine.

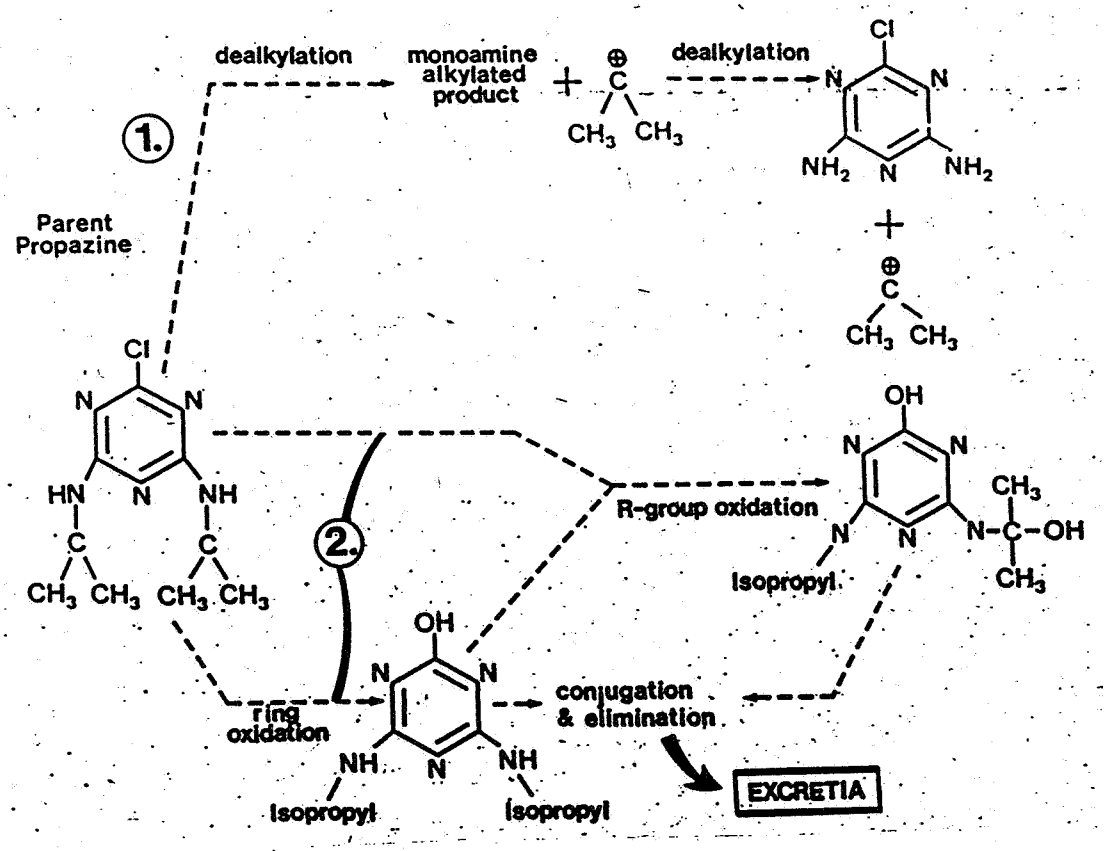
There were no compound-related effects on mortality, clinical signs, body weight, food consumption or gross pathology. Hematology, urinalysis, clinical chemistries, and organ weights were not determined. At 3000 ppm, an increased incidence of myocardial degeneration was observed in the female mice (17/59 vs 4/60 in controls) and an increased incidence of hemosiderin-laden macrophages was observed in the livers of male mice (15/59 vs 3/60 in controls). At the doses tested, there was not a treatment-related increase in tumor incidence. **The NOEL is 1000 ppm (150 mg/kg/day) and the LEL is 3000 ppm (450 mg/kg/day) based on myocardial degeneration in females and hemosiderin-laden macrophages in the livers of males.**

The dosing in this study was considered to have been adequate.

E. Additional Toxicology Data on Propazine

1. Metabolism of Propazine

There is no acceptable metabolism study. However, based on open literature information, the general metabolic pathway of propazine in the rat is shown below:



2. Mutagenicity

a. Gene Mutation (MRID # 00163222; HED Doc. #s 005611 and 005823)

Propazine was tested in V79 Chinese hamster cells with and without microsomal activation. Propazine produced a dose-related positive mutagenic response without metabolic activation and a weak (nondose-related) positive response with activation.

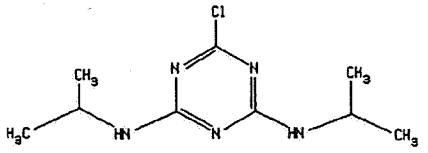
b. Nucleus Anomaly Assay (MRID # 00150622; HED Doc. #s 005226 and 005823)

Groups of six male and six female Chinese hamsters were orally administered propazine at dosages of 0, 1250, 2500, or 5000 mg/kg on 2 consecutive days. The cells displaying anomalies of nuclei in treated cells did not differ significantly from the negative controls. Propazine was not considered mutagenic in this assay.

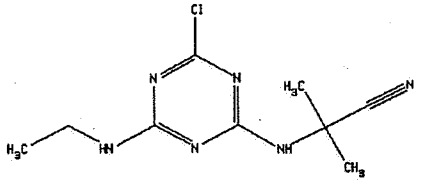
c. DNA Damage and Repair (MRID # 00150623; HED Doc. #s 005226 and 005823)

Assay for unscheduled DNA synthesis was rat hepatocytes were performed with concentrations of 0, 0.5, 2.5, 12.5 and 62.5 ug/mL. The mean number of silver grains per nucleus in the vehicle control and treated cells (at any dose level) was not markedly different. Propazine was not mutagenic for DNA damage and repair under the conditions of this assay.

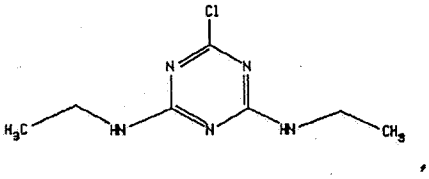
4. Structure-Activity Relationships



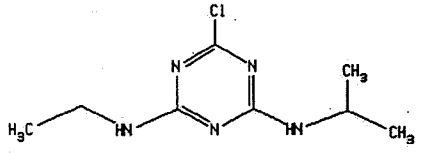
Propazine



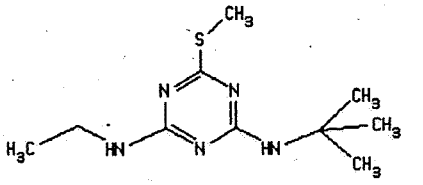
Cyanazine



Simazine



Atrazine



Terbutryn

Cyanazine was negative in CD mice up to 1000 ppm, but produced a statistically significant increase in malignant mammary gland tumors (adenocarcinoma, carcinosarcoma) in female Sprague-Dawley rats at 25 and 50 ppm. The increased incidence had a significant trend and pairwise comparison and was outside the range of historical controls. Cyanazine was genotoxic in the mouse lymphoma gene mutation assay and unscheduled DNA synthesis in rat hepatocytes. Cyanazine was classified as Group C carcinogen with quantification of human cancer risk using a low-dose extrapolation model (Q_1^*).

Simazine was associated with significant increases in carcinomas of the pituitary gland at 1000 ppm (HDT) and carcinomas of the mammary gland at the mid dose of 100 ppm and the high dose of 1000 ppm in Sprague-Dawley rats. Simazine was not associated with an increase in neoplasms in CD-1 mice up to 4000 ppm. There are both positive and negative results in the Ames assay in the published literature. Positive results are reported in the mouse lymphoma, Drosophila sex-linked recessive lethal and cell transformation assays. Simazine was classified as a Group C carcinogen with quantification of human risk using low-dose extrapolation model (Q_1^*).

Atrazine was associated with a significant increase in mammary gland fibroadenomas at 1000 ppm, in mammary gland adenocarcinomas at 70, 500, and 1000 ppm and in total mammary gland tumor-bearing animals at 1000 ppm in female Sprague-Dawley rats. Atrazine was not carcinogenic when tested in CD-1 mice. Atrazine was negative for genotoxicity in three acceptable assays, although there are some positive results in the published literature including mouse bone marrow aberrations and a mouse dominant lethal assay. Atrazine was classified as a Group C carcinogen with quantification of human risk using low-dose extrapolation model (Q_1^*).

Terbutryn induced a significant increase in combined mammary gland adenomas and carcinomas and combined hepatocellular adenomas and carcinomas in female Sprague-Dawley rats. In males, terbutryn induced an increase in combined thyroid follicular cell adenomas and carcinomas and in testicular interstitial cell adenomas. Terbutryn is negative for carcinogenicity in CD-1 mice and is negative in the Ames assay, chromosomal aberrations <u>in vivo</u> in hamsters, and the micronucleus assay. Terbutryn has been classified as a Group C carcinogen.

F. Weight-of Evidence Consideration

The Committee considered the following facts regarding the toxicology data on propazine in a weight-of-the-evidence determination on carcinogenic potential.

1. The decreased body weight and weight gain in both sexes (♂, 10-13% decrease in BW and 16% decrease in BW gain; ♀, 7-11% decrease in body weight and 16-27% decrease in weight gain)) at the high dose is considered toxicologically significant and evidence that adequate high dose levels in both sexes were used to assess carcinogenicity.

2. Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls, for mammary gland adenomas, and adenomas and/or adenocarcinomas combined, all at $p < 0.01$. There was also a significant increasing trend, and significant differences in the pair-wise comparisons of the 3 and 1000 ppm dose groups with the controls, for mammary gland adenocarcinomas, all at $p < 0.05$. In addition, there was a significant difference in the pair-wise comparison of the 3 ppm dose group with the controls for mammary gland adenomas and/or adenocarcinomas combined at $p < 0.01$. There were no statistically significant increases in mammary gland fibroadenomas. The propazine study was conducted between 1976 -1978. The incidence of adenocarcinomas in the 1000 ppm propazine treated group (24%) exceeded the 20 of the 22 historical control studies and at 3 ppm, the incidence of adenocarcinomas in the propazine treated group (22%) exceeded 19 out of 22 historical control studies. With respect to adenomas, the incidence in the 1000 propazine treated group (17%) exceeded 16 out of 18 historical control studies.

3. Propazine was negative for neoplasms in CD-1 mice at doses up to 3000 ppm.

4. Propazine was tested in V79 Chinese hamster cells <u>with</u> and <u>without</u> microsomal activation. Propazine produced a dose-related positive mutagenic response <u>without</u> metabolic activation and a weaker (nondose-related) positive response with activation. Propazine was negative in a nucleus anomaly test and in an unscheduled DNA synthesis assay.

5. Propazine is structurally related to other s-triazines which produce mammary gland tumors and includes terbutryn, atrazine, cyanazine, and simazine.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for Propazine.

The Peer Review Committee agreed that Propazine should be classified as a Group C - possible human carcinogen and that a low-dose extrapolation methodology (Q_1*) be applied to the animal data. This decision was based on evidence of increased incidences of mammary gland adenomas, carcinomas and combined adenoma/carcinoma in the female Sprague-Dawley rat, by both pair-wise and trend analysis, at a dose that was adequate and not excessive. The incidences of the tumors exceeded the means of the historical controls. Positive results in a gene mutation assay with Propazine, and information from structural analogs of Propazine (Cyanazine, Simazine, Atrazine, Terbutryn) which also induce tumors at the same site (mammary gland) in rats, provided additional support.

The CPRC recommended that as a follow-up to the positive gene mutation assay, according to the guidelines for mutagenicity, an interaction with gonadal DNA study be performed by the registrant.

For the purpose of risk characterization, the CPRC recommended that the quantification of human risk (Q_1^*) for Propazine be based on the total mammary gland tumors (adenomas and carcinomas combined) in the female rat.

It should be noted that the evaluation of Propazine will be revisited in the near future, as part of a consideration of the Triazines as a class.