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FINAL

DATA EVALUATION REPORT

Prometryn Technical

Study Type: Oncogenicity in Mice

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

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Principal Author: John D. Liccione Date 4/23/92
John Liccione, Ph.D.

Reviewer: William McLellan Date 4-23-92
Bill McLellan, Ph.D.

QA/QC Manager: Sharon A. Segal Date 4/23/92
Sharon Segal, Ph.D.

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Project Officer: Mr. James Scott

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EPA Reviewer: Myron S. Ottley, Ph.D.
Review Section IV, Toxicology Branch I,
Health Effects Division

Signature: MSOttley
Date: 4/21/93

EPA Section Head: Marion Copley, D.V.M.
Section Head, Review Section IV,
Toxicology Branch I, Health Effects Division

Signature: Marion Copley
Date: 4/29/93

DATA EVALUATION REPORT

STUDY TYPE: Guideline Series 83-2: Oncogenicity study in mice.

TEST MATERIAL: Prometryn technical

SYNONYM: Prometryne

MRID Number: 404662-01

PC Number: 80805

Tox. Chemical Number: 0.097

STUDY NUMBER: 438-128

SPONSOR: CIBA-GIEGY Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, VA 22180

TITLE OF REPORT: 102-Week Carcinogenicity Study in Mice: Prometryn Technical Final Report

AUTHOR: Walter Kundzins

REPORT ISSUED: March 16, 1981

QUALITY ASSURANCE: A quality assurance statement was signed but not dated.

CONCLUSIONS: Prometryn was fed to male and female CD-1 mice at dietary levels of 0, 10, 1000, or 3000 ppm (corresponding to approximately 0, 1, 100, or 300 mg/kg/day).

NOEL for females = 1000 ppm

LOEL for females = 3000 ppm, based on decreases in body weight gains.

Mean body weight gains in the high-dose females were significantly ($p \leq 0.05$) lower than those of controls during the first 48 weeks of the study. There was no significant effect of dosing on clinical signs, mortality, gross

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pathology or histopathology. Prometryn was not oncogenic under the conditions of the study.

Based on decreased mean body weight gain in the high-dose females, adequate toxicity to evaluate carcinogenic potential of the test material was achieved.

CORE CLASSIFICATION: Core Supplementary for an Oncogenicity evaluation (83-2) in mice. This study is not upgradable by itself since adequate toxicity to evaluate carcinogenic potential of the test material was not achieved in males. Homogeneity analyses of the test material in the diets were not performed Other deficiencies are listed on page 13.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Prometryn technical

Batch number: FL-761355 (3 samples)

Purity: Not reported

Physical property: White powder with small lumps

Storage: Room temperature

Stability: Not reported

2. Test Article Analyses for Purity and Stability

Test diets were prepared by the addition of test substance to a small amount of basal diet and blended in a Waring mixer to produce a premix. Additional basal diet was added until the desired concentration of the test substance in the feed was reached. Mixing time for diets was one minute/kg of diet. Control mice received the basal diet only. Test diet mixtures were prepared weekly. For each weekly diet preparation, 100 g samples were taken, frozen and shipped to the study sponsor at approximately quarterly intervals for the first year, and then at 4-week intervals thereafter. Approximately 1 g of the test material was shipped with the diet samples.

The results of dietary concentration analyses and stability analyses were presented in the "residue recovery report" appended to the study report. Dietary concentration analyses of the test material in mouse chow were performed at weeks 13, 26, 39, 52, 56, 60, 64, 69, 74, 78, 82, 86, 91, 95, 99 and 102. Stability of the test material was determined in rat chow rather than mouse chow; stability was determined for up to 14 days. The homogeneity of the test material in diets was not examined.

Results of stability analyses indicated that the test material was stable in rat chow for up to 14 days at room temperature. Mean concentrations of the test material in diets at dose levels of 10,

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1000, and 3000 ppm were 119%, 106.8%, and 103.3% of target, respectively.

3. Animals

Weanling albino Ham/ICR Swiss, Charles River CD®-1 mice (462 males and 462 females) were received from Charles River Breeding Laboratories, Wilmington, MA. The mice were acclimated to laboratory conditions and diet one week prior to the initiation of treatment. After the acclimation period, all mice were examined by a staff veterinarian to determine health status and 480 of the animals were selected for study. Test animals were housed in groups of five of the same sex in polycarbonate boxes. Animals were uniquely identified by ear clippings. At initiation of treatment, males weighed 12-35 g and females weighed 11-27 g. Food (Purina Laboratory Chow) and tap water were provided ad libitum. It was assumed by the study author that there were no known contaminants in the basal diet and drinking water likely to interfere with the study. Temperature was maintained between 67-76°F throughout the study. Humidity ranged between 25%-77% during the study period.

Animals were assigned by sex to the following test groups using a table of random numbers:

Dietary Levels (ppm)	Number of Animals	
	Males	Females
0	60	60
10	60	60
1000	60	60
3000	60	60

Examination of body weight data indicated that the animals were not randomized to respective dose groups on the basis of body weights, since the mean body weights of the mid- and high-dose groups were lower than that of the controls at the initiation of dosing.

Dose Selection: Although a rationale for dose selection was not discussed in the study report, a range-finding study in mice (Piccirillo, 1977. 28-Day Pilot Feeding Study in Mice: Prometryn Technical - Final Report, Hazleton Laboratories America, Inc., MRID 00404575-15) was available for review. In the range-finding study, groups of five male and five female mice were fed diets containing 30, 100, 300, 600, 1,000, 3,000, 10,000, and 30,000 ppm prometryn for 28 days. Four of the high-dose mice died during the first week; the remaining high-dose animals were dead by the end of the second week. Clinical signs observed in the surviving high-dose animals at the end of the first week included hunched appearance, thinness, labored respiration, and urine stains on the fur. Hunched appearance was

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also observed at the end of week 1 in four of the animals fed 10,000 ppm. Moderate-to-marked decreases in body weight were noted in the animals receiving 10,000 or 30,000 ppm during the first week of the study. Body weight gain in mice receiving 10,000 ppm exceeded that of controls during the remainder of the study. Treatment-related gross findings in some of the high-dose animals that died during the study consisted of reddening of the mucosal surface of the gastrointestinal tract and the presence of dark-colored contents in the gastrointestinal tract. At terminal sacrifice, the mucosa of the small intestine from three animals receiving 10,000 ppm were characterized as dark pink in color.

4. Statistics

Body weight data as presented in the original study report were analyzed by the Games and Howell's modification of the Tukey-Kramer multiple pairwise test. In a subsequent addendum to the study report, body weight data were re-analyzed by analysis of covariance (ANCOVA) using the pretreatment body weights as the covariate, since results of analysis for variance (ANOVA) for week 0 revealed statistically lower mean body weights for the low-, mid-, and high-dose males and females. Dunnett's t-test was utilized to compare treatment groups with the control group if the ANCOVA revealed significance. Survival data (to week 100) were evaluated by a life table technique. No statistical analysis was conducted on food consumption data due to apparent food wastage. There was no indication that pathological data were examined statistically.

5. General Observations

(a) Mortality/morbidity/survival

All of the animals were observed twice daily for mortality and moribundity up to 100 weeks of the 102-week treatment period. The numbers of scheduled deaths (SD) and unscheduled deaths (UD) occurring during the study were reported for each dose group as follows:

Group	Male		Female	
	SD	UD	SD	UD
0 ppm	16	44	27	33
10 ppm	12	48	22	38
1000 ppm	14	45[a]	21	39
3000 ppm	14	46	26	34

[a]Number excludes one male that was killed accidentally.

Survival of all treated groups of both sexes was generally comparable to controls. At approximately 18 months of treatment, the survival rate for males was approximately 83, 67, 83 and 53% in the control, low-, mid-, and high-dose groups, respectively.

For females, it was approximately 85%, 88%, 85%, and 87% in the control, low-, mid-, and high-dose groups, respectively. Statistical analysis at week 100 showed no statistically significant differences in the incidence of mortality in treated animals compared to controls.

(b) Clinical observations

Detailed clinical observations for gross signs of toxicity were conducted every four weeks. Observations for the incidence and location of palpable masses, tissue masses, and wart-like lesions were also made every four weeks. Although the study author indicated that the size of palpable masses was also recorded, no data could be located.

There were no effects of dosing on the incidence of clinical signs. Incidental findings in control and treated animals of both sexes included hunched and/or thin appearance, staining and/or roughening of the haircoat, and bloating. There was no effect of dosing on the incidence of palpable nodules, tissue masses, and wart-like lesions.

(c) Body weight/food consumption/compound intake

Body weight. Individual body weights were recorded every four weeks.

The study authors indicated (on page 14 of the study report) that mean body weights of the low-, mid-, and high-dose males and high-dose females were statistically significantly (level of statistical significance not reported) lower than those of controls at week 0. As a result, post hoc analysis of body weight data (presented as an addendum to the original study report) was performed using ANCOVA (to adjust the treatment means for differences attributable to initial body weight differences) followed by Dunnett's t-te. However, intergroup covariance adjusted treatment mean body weights calculated by ANCOVA were not presented in the addendum; only the levels of statistical significance between treatment versus control groups were reported. The results of the statistical analysis of the body weight data are summarized in Table 1. Although the addendum mentions that mean adjusted body weights of the mid- and high-dose females were consistently lower than the control values only during the first 32 weeks of treatment, the results of Dunnett's t-test indicate that the decreases were consistent during the first 48 weeks of treatment (with the exception of week 8). Results of post hoc statistical analysis of body weight data revealed sporadic decreases in mean adjusted body weights in the mid- and high-dose males during the first 16 weeks of treatment.

Mean body weight gain data (based on unadjusted or covariance adjusted mean body weight values) were not provided in the original study report or in the addendum to the study report.

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TABLE 1. Summary of Mean Body Weight (g ± SD) in Mice Fed Prometryn Technical For 102 Weeks[a]

Dietary Level (ppm)	Summary of Mean Body Weight at Weeks: 0	12	28	52	76	100[b]
			Males			
0	23 ± 4.1	37 ± 2.8	40 ± 3.6	40 ± 3.5	40 ± 3.0	38 ± 5.7
10	21 ± 3.2	35 ± 2.8	39 ± 3.6	39 ± 3.5	37 ± 5.2	40 ± 3.6
1000	20 ± 3.2	35 ± 2.9**	38 ± 3.7	39 ± 3.6	39 ± 5.4	39 ± 6.1
3000	19 ± 2.8***	34 ± 4.3**	39 ± 4.7	37 ± 3.5	38 ± 3.5	36 ± 4.2
			Females			
0	20 ± 3.4	30 ± 2.4	34 ± 3.8	34 3.6	34 ± 3.4	37 ± 3.6
10	20 ± 2.4	28 ± 2.8**	33 ± 3.6**	35 ± 4.1	31 ± 5.0**	33 ± 4.9**
1000	19 ± 2.4	28 ± 2.8**	32 ± 3.2**	33 ± 4.3	33 ± 3.7	33 ± 4.0**
3000	17 ± 2.1***	27 ± 2.8**	30 ± 3.0**	31 ± 3.3	30 ± 4.5**	32 ± 4.7**

[a]Unadjusted mean body weight data were extracted from Table 1 of the study report; results of statistical analysis of body weight data were extracted from Table 1 of Amendment I.

[b]Mean body weights were not recorded after week 100.

* Significantly different from control group ($p < 0.05$) based on ANCOVA and Dunnett's t-test.

** Significantly different from control group ($p < 0.01$) based on ANCOVA and Dunnett's t-test.

*** Significantly different from control group ($p < 0.01$) based on ANOVA and Dunnett's t-test.

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TABLE 2. Summary of Mean Body Weight Gain (g) For Selected Intervals In Mice Fed Prometryn Technical For 102 Weeks[a,b]

Dietary Level (ppm)	Mean Body weight Gain (g) at Weeks: 16	48
	Males	
0	17.46	20.13
10	16.91	19.00
1000	17.68	20.30
3000	17.11	19.52
	Females	
0	11.49	15.41
10	11.05	14.98
1000	10.98	14.83
3000	9.99	13.76

[a]Mean body weight gain data calculated by the Reviewers.
[b]Mean body weight gains represent adjusted mean values calculated by Analysis of Covariance utilizing the PROC GLM program of SAS/STAT software.

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Since adjusted treatment mean body data (means adjusted for the covariate) were not presented in the study report, the reviewers evaluated mean body weight gain data by ANCOVA; covariance analysis was performed utilizing the PROC GLM program of the SAS/STAT software. Post hoc analysis of the data was performed with Dunnett's test if the ANCOVA revealed any significance between treated and control groups. Table 2 summarizes mean body weight gain data evaluated during the first 16 weeks (the time interval at which statistically significant decreases in mean body weights were noted in the mid- and high-dose females and in the high-dose males), and 48 weeks (the time interval that the reviewers noted statistically significant and consisent decreases in mean body weights in the mid- and high-dose females). Mean body weight gains in the low-, mid-, and high-dose females were approximately 3.8, 4.4, and 13.1%, respectively, lower than that of control at week 16. Mean body weight gains in females receiving the same dose levels were 2.8, 3.8, and 10.7%, respectively, lower than that of control at week 48. Mean body weight gains in males receiving the same dose levels were comparable to that of control at both weeks 16 and 48. The results of ANCOVA indicated that the decreases in mean body weight gains in the high-dose females at weeks 16 and 48 were not statistically significant.

Food consumption and compound intake. Individual food consumption was determined every four weeks by dividing the total consumption value per cage by the number of animals alive in the cage at the beginning of the feeding period. Individual compound intake was calculated at the same intervals as food consumption calculations (presented in Appendix 6 of the Study Report).

Table 3 summarizes selected data on food consumption. In general, food consumption was slightly higher in the treated males and females compared to those of controls. Statistical analysis of food consumption data was not conducted because of food wastage. Mean compound intakes were not tabulated in the study report. However, based on a conversion factor of 0.1 mg/kg/day = 1 ppm, the compound intakes were approximately 1, 100, or 300 mg/kg/day.

(d) Ophthalmological examinations:

Ophthalmological examinations were not performed.

6. Clinical Pathology

Hematology, clinical chemistry, and urinalysis parameters were not measured.

7. Sacrifice and Pathology

Following 102 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital, and exsanguinated. Necropsies were performed on all of the animals sacrificed at termination and those that died or were sacrificed moribund during the study. The

TABLE 3. Mean Food Consumption (g/animal/week) at Selected Intervals for Mice Fed Prometryn Technical for 102 Weeks[a]

Dietary Level (ppm)	Summary of Mean Food Consumption at Weeks:					
	4	12	28	52	76	100[b]
			Males			
0	35	37	41	34	35	31
10	37	39	40	37	36	37
1000	37	40	42	38	37	40
3000	37	41	41	41	42	38
			Females			
0	37	37	38	30	33	33
10	35	38	39	35	32	28
1000	38	38	38	37	35	34
3000	38	43	41	38	34	30

[a]Data extracted from Table 1 of the study report.
[b]Mean food consumption was not recorded after week 100.

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checked (X) organs were preserved in 10% neutral buffered formalin. Organ weights were not determined.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	X Brain
X Salivary glands*	X Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord (three levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen	X Eyes (Optic nerve)
X Jejunum*	Thymus*	
X Ileum*		
X Cecum*	Urogenital	
X Colon*		
Rectum	X Kidneys*	Glandular
X Liver*	X Urinary bladder*	
X Gallbladder*	X Testes*	X Adrenals*
X Pancreas*	X Epididymides	Lacrimal gland
	X Prostate	X Mammary gland
Respiratory	X Seminal vesicle	X Thyroid*
	X Ovaries	X Parathyroid*
X Trachea*	X Uterus	Harderian glands
X Lung*	X Vagina	

Other

X Bone (sternum and femur)
X Skeletal muscle*
X Skin
X All gross lesions and masses

* = Recommended by Subdivision F (November 1984) Guidelines

Most tissues of control and high-dose groups were stained with hematoxylin and eosin and examined histologically for the animals sacrificed at termination, and for those that died or were sacrificed in a moribund condition. In addition, any unusual lesions and suspect tumors in the low- and mid-dose animals were examined histologically. Tissue masses were examined in all animals that were sacrificed at termination, and in all animals that died (except one mid-dose male killed accidentally) or were sacrificed in a moribund condition during the study.

(a) Macroscopic

There were no compound-related gross observations among the animals administered prometryn.

Autolysis and/or cannibalization in a number of dead and moribund sacrifice animals precluded a complete necropsy in these animals. The severity of autolysis ranged from slight to very advanced. The incidences of autolysis in males fed 0, 10, 100, or 3000 ppm were 5 of 44, 5 of 48, 6 of 45, and 7 of 45 males, respectively. The incidences of autolysis in females receiving the same dietary

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levels were 3 of 33, 5 of 38, 4 Of 39, and 4 of 34 females, respectively. The digestive tract was the tissue most affected by autolysis. Other tissues significantly affected by autolysis included the spleen, pituitary gland, pancreas and lymph nodes. The incidences of cannibalization in males fed 0, 10, 100, or 3000 ppm were 2 of 44, 1 of 48, 4 of 45, and 3 of 45 males, respectively. The incidences of cannibalization in females receiving the same dietary levels were 3 of 33, 0 of 38, 5 of 39, and 4 of 34 females, respectively.

(b) Microscopic

Nonneoplastic. There were no neoplastic findings in males and females attributed to administration of prometryn.

The addendum to the study report presented a summary tabulation of the incidence of nonneoplastic findings in dead and moribund sacrifice animals and in terminal sacrifice animals and combined incidences for all animals. Individual histopathology data were presented. The most frequent finding in dead and moribund animals and in terminal sacrifice animals was amyloidosis. The incidence of amyloidosis in treated animals of both sexes was comparable to those of controls. Amyloidosis was present in nearly all organs; however, amyloid deposition was seen mostly in the small intestines, kidney, liver, thyroid, adrenal and salivary glands, stomach, ovaries, and the heart. The severity of the amyloidosis was not described. The incidence of other findings commonly seen in mice was similar in the treated and control animals. These findings were noted in moribund and dead animals and in terminal sacrifice animals and included pulmonary perivascular and peribronchiolar lymphoid proliferation, chronic renal disease, mild pneumonitis, inflammatory changes in the liver, and hyperplasia of lymphoid organs.

Neoplastic. There were no neoplastic lesions attributed to administration of prometryn.

Summary tabulation of tumor indicence were presented separately for dead and moribund animals and for terminal sacrifice animals. Several neoplastic lesions that were considered by the study author to be age-related were noted in these animals. The most frequent neoplastic lesion observed in the dead and moribund males and in the terminal sacrifice males was hepatocellular carcinoma. The total incidences of hepatocellular carcinoma (combining data for dead and moribund males and terminal sacrifice males) in male mice fed 0, 10, 1000, or 3000 ppm prometryn in the diet were 6 of 60, 4 of 35, 7 of 34 and 4 of 60 males, respectively. Other neoplastic lesions seen in both dead and moribund males and in terminal sacrifice males included hepatocellular adenoma, and pulmonary bronchiolar/alveolar carcinoma and/or adenoma. The most frequent neoplastic lesions noted in dead and moribund females and terminal sacrifice females were lymphoid neoplasms. The total incidences of lymphoid neoplasms (combining data for dead and moribund females and terminal sacrifice females) in female mice fed 0, 10, 1000, or

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3000 ppm prometryn were 4 of 60, 9 of 33, 7 of 33, and 8 of 60 females, respectively. The study author indicated that the lymphoid lesions were within normal historical limits for aged female mice of this strain; however, historical control data were not provided. Other neoplastic lesions observed in dead and moribund females or in terminal sacrifice females included pulmonary bronchiolar/alveolar carcinoma and/or adenoma, and hepatocellular carcinoma or adenoma.

B. REVIEWERS' DISCUSSION

This study was conducted and completed prior to the effective date of the GLP Guidelines. The sponsor submitted an addendum to the study report providing a statistical re-analysis of body weight data; a summary tabulation of nonneoplastic lesions in dead and moribund sacrifice animals, and in terminal sacrifice animals; the combined incidences of nonneoplastic lesions in all animals; and corrections to certain individual histopathology data. However, the reviewers noted several deficiencies in the study. Deficiencies are as follows:

1. Animals were not randomly assigned to dose groups with respect to body weight. As a result, initial mean body weights were slightly lower in the treated males and females compared to control animasl. To adjust for this baseline difference in body weights, the sponsor re-analyzed body weight data by analysis of covariance (ANCOVA) using the pretreatment body weights as the covariate (results presented in the addendum to the study report). Dunnett's t-test was utilized to compare treatment groups with the control group if the ANCOVA revealed significance. However, the covariance adjusted mean body weight values were not presented in the addendum; only levels of statistical significance were presented. In addition, there were inconsistencies in the reporting of the statistical significance of some of the body weight data in the addendum. For example, the results of ANCOVA indicated that there were statistically significant differences in mean body weights in treated females at week 52, and in treated males at weeks 64 and 72, but the results of Dunnett's test for these animals at respective weeks were not presented to identify which treated groups were different than controls.

2. Homogeneity of the test material in the diets were not performed.

3. A number of animals (at least 10% in control and in all treatment groups of both sexes) were lost to autolysis and/or cannibalism. The autolysis and cannibalism precluded a thorough pathological examination in these animals.

4. Individual food consumption values should be reported as estimated values since individual consumption was calculated by dividing the total consumption value per cage by the number of animals alive in the cage at the beginning of the feeding period. Statistical analysis of food consumption data was not performed because of food wastage. The lack of accurate food consumption values makes it difficult to correlate changes in individual food intake with changes in individual body weight. Also, individual food consumption is useful for health monitoring purposes.

5. A blood smear was not obtained from 10 animals/sex/dose group at 12 months, 18 months, and at sacrifice, as required by the Guidelines.

6. Organ weights were not determined.

7. A quality assurance statement was signed, but not dated.

8. Dose selection was inadequate in males. Based on the results of the 28-day study it appears that 10,000 ppm, or at least 5000-7000 ppm, may have been more appropriate.

Based on decreased mean body weight gain (covariate adjusted means calculated by the reviewers) in the high-dose females, adequate toxicity to evaluate the carcinogenic potential of the test material was achieved. The reductions in mean body weight gains in the hign-dose females were for the most part consistent during the first 48 weeks of the study.

There was no oncogenic effect under the conditions of the study.

There were no toxicologically significant changes in mortality, clincial signs, gross pathology, or histopathology.

The most frequent nonneoplastic finding was amyloidosis, which was present in nearly all organs and occurred with similar incidence in treated and control animals. The severity of the amyloidosis was not reported and the degree to which this lesion affected the pathological evaluation of tissues is difficult to assess. Common spontaneous neoplasms included hepatocellular neoplasms, lymphomas, and alveolar/bronchilar tumors of the lung.

The reviewers assess that the LOEL in females is 3000 ppm based on decreased body weight gain; the NOEL is 1000 ppm. A LOEL for males was not established.

RM# 0615-00

MRID# 40466201 (DERs)

Page_____ is not included in this copy.

Pages 15 through 18 are not included.

The material not included contains the following type of information:

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_____ Description of the product manufacturing process.

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_____ The product confidential statement of formula.

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