



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

3-8-84

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Tox Chem. No. 96

DATE:

SUBJECT: Prometon - IBT Replacement: Rat and Rabbit
Teratology Studies and 90-Day Rat Feeding Study. EPA I.D. #100-544.

TO: Robert Taylor (PM #25)
Registration Division (TS-767C)

THRU: William B. Butler, Jr., Head
Review Section III
Toxicology Branch (TS-769C)
Hazard Evaluation Division

William B. Butler 3/8/84

and

William Burnam, Chief
Toxicology Branch (TS-769C)

Applicant: Ciba-Geigy Corporation
Agricultural Division
P.O. Box 11422
Greensboro, NC 27409

Applicant: International Flavors & Fragrances, Inc.
521 West 57th Street
New York, New York 10019

Action Requested:

Review of the IBT-replacement data, which were submitted by the applicant, was requested by the Registration Division.

Comments:

- (1) The IBT-replacement studies have been reviewed and evaluated. The ratings have been assigned as "Minimum" and "Supplementary" as indicated.
- (2) The specific reference or literature on the sensitivity of the Charles River Crl:COB5^{CD}(SD)BR rats to the teratogenic effects of Prometon Technical are required to be submitted in order to support their claim or statement made in the teratogenicity study of Prometon Technical in pregnant rats (Argus Project 203-003, dated April 10, 1981).

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- I. Teratogenicity Study of Prometon Technical in Pregnant Rats by M. Christine Florek, Gareth D. Christin, Mildred S. Christin and E. Marshall Johnson of Argus Research Laboratories, Inc., Perkasie, Pa.; Argus Project 203-003, dated April 10, 1981.

A. Experimental

(1) Prometon Technical (Batch FL 800793) was provided by Ciba-Geigy Corporation as a white powder. Suspensions of this test material in corn oil (Mazola[™], lot No. APR 23 81 A) were prepared daily at concentrations of 36, 120 and 360 mg/ml.

(2) "The Charles River Crl:COB5[™]CD[™](SD)BR rats were used because this species and strain has been shown to be sensitive to teratogenic effects of compounds."@ The virgin female rats were 53 days old on arrival at the laboratory. The mating processes with the male rats of the same strain followed an acclimation period of about 2 weeks. The male rats were 90 days old when received at the laboratory.

(3) After mating, one hundred female Charles River rats, which were naturally bred and randomly assigned to four experimental groups (25 rats/group), were given orally via gavage the suspensions of Prometon Technical in corn oil with concentrations of 0(Control), 36, 120 and 360 mg/ml at volumes of 1 ml/kg/day.

(4) 25 rats in each of four treatment groups were given the aforementioned oral dosages of Prometon Technical or vehicle once daily on days 6 through 15 of presumed gestation. Daily adjustments in dosages administered were made on the basis of maternal body weight.

(5) The treated rats were observed for toxic effects, abortion or death. Body weights were recorded throughout the experiment. On day 20 of presumed gestation, the female rats were killed with CO₂ and the abdomen of each was opened and examined for pregnancy and number and placement of implantations, early and late resorptions, live and dead fetuses and number of corpora lutea. In the case of fetuses, each was individually weighed, sexed and examined for any maternal abnormalities. Furthermore, the live fetuses were killed, and one-third of each litter examined for soft tissue anomalies using a variation of Wilson's sectioning technique. Two-thirds of the fetuses in each litter were cleaned, stained and examined for skeletal variations.

(6) The statistical tests, such as Bartlett's test of homogeneity of variance, Durnett's test, Kruskal-Wallis test, the Fmax test and others, were used in this study.

B. Results

(1) No mortality occurred during the study.

@Note: This was quoted from the applicant's statements, but no specific reference was given to support the claim. Further, the applicant also stated that "historical data are available in the literature", again no source of the literature was mentioned.

(2) The excess salivation in 36, 120 and 360 mg/kg/day dosage group rats, chromorrhinorrhea in 120 and 360 mg/kg/day dosage group rates and other signs, such as chromodacryorrhea, decreased motor activity, urine-stained abdomen, lacrimation, increased respiration rate, lost or impaired righting reflex, ptosis and pilo-erection, showed in 360 mg/kg/day dosage group rats, are considered as the treatment-related antemortem physical signs.

(3) There was no postmortem physical signs which might be considered as relating to the treatment of pregnant rats with the test chemical.

(4) The treated pregnant rats in the 120 or 360 mg/kg/day dosage groups gained less body weight compared with the vehicle controls during the treatment period (days 6 through 15 of gestation) and throughout gestation (days 0 through 20). However, body weight gain in 36 mg/kg/day dosage group rats was close to that of vehicle controls throughout the test.

(5) Pregnancy occurred in 23, 24, 24 and 22 rats giving 0 (vehicle), 36, 120 and 360 mg/kg/day of Prometon Technical respectively. Caesarean-sectioning observations on day 20 of gestation were based on these respective numbers of rats because no rat died during the study.

(6) Treatment of pregnant rats with the test compound did not affect implantation, litter size, fetal viability and resorption in significant degrees at the dose levels administered in the experiment. The percentage of male to female fetuses was slightly higher among the 360 mg/kg/day group than among vehicle controls, but this observation may not be considered as treatment related.

C. Conclusion

According to the aforesaid data, Prometon Technical did not appear to be teratogenic to Cr1:COBS "CD", BR rats when administered orally via gavage on days 6 through 15 of gestation at dosages of 36, 120 and 360 mg/kg/day.

Core classification: Minimum

- II. Teratogenic potential of Prometon Technical in New Zealand White Rabbits (Segment II Evaluation by Gerald E. Lightkep, Mildred S. Christian, Gareth D. Christian and E. Marshall Johnson of Argus Research Laboratories, Inc., Harsham, Pennsylvania, 19044; Argus Protocol 203-002; Final report date: June 3, 1982).

A. Experimental

(1) Prometon Technical (Batch FL No. 800793 - 80% active, 20% inerts), a white powder, was provided by Ciba-Geigy Corporation). Corn oil (Mazola, Lot June 23, 1981 A) was used as the vehicle for all suspensions.

(2) Following an acclimation interval of about four weeks, 64 virgin female DLI:NZW rabbits (from Dutchland Labs, Denver, Pennsylvania), which were approximately 6.5 months old and with body weights ranging from 3.5 - 4.9 kg each were selected for the study. These rabbits were intravenously administered 20 USP units/kg of HCG (PREGNYL[™], Organon, Inc., Lot 182179311, diluted to 200 USP units/ml of normal saline) approximately 3 hours prior to insemination with an estimated 0.25 ml of semen which was diluted with normal saline to a concentration of 6.0×10^6 spermatozoa/ 0.25 ml. The day of artificial insemination was designated day zero of presumed gestation. 16 female rabbits were inseminated on each of four consecutive days.

(3) Suspensions of Prometon Technical were prepared daily using corn oil. All suspensions were prepared at concentrations appropriate for administration to rabbits in all dosage groups at ^{1.0 ml/kg/day} 1.0 ml/kg/day (with dosages of 0 (vehicle), 0.5, 3.5 and 24.5 mg/kg/day). The test material was administered orally via stomach tube to female rabbits once each day on days 6 through 13 of presumed gestation to 18, 16, 16, and 16 artificially inseminated rabbits in each of the four respective treatment groups.

(4) Rabbits were observed for signs of agent effect, abortion and delivery. On day 30 of presumed gestation, female rabbits were killed with CO₂, and the abdomen of each was opened and examined for pregnancy and number and placement of implantations, early and late resorptions, live and dead fetuses and number of corpora lutea.

(5) Maternal body weight data, fetal body weight data, fetal anomaly data and other related data were analyzed statistically.

B. Results

(1) There was no maternal death which was attributed to the treatment of rabbits with the test material which occurred during this study, although one pregnant rabbit from the 0.5 mg/kg/day group became partially paralyzed due to result of a back injury.

(2) Pregnancy occurred in 16, 13, 13 and 11 rabbits who received 0 (vehicle), 0.5, 3.5 and 24.5 mg/kg/day dosages of prometon technical, respectively.

(3) Four animals aborted (one control doe; one at 0.5 mg/kg/day and two at 24.5 mg/kg/day), but there was no apparent increase in abortion as the result of administration of Prometon Technical to the rabbits.

(4) It was observed that anorexia and excess lacrimation occurred more frequently in rabbits receiving high doses (3.5 and 24.5 mg/kg/day groups) as compared with controls and low dose groups (0.5 mg/kg/day). These abnormalities were attributed to a toxic effect of the test material.

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(5) Maternal body weight gain in the animals treated with Prometon at 24.5 mg/kg/day was significantly reduced from day 6 to 18 of gestation as compared with the control. However, the effect disappeared after cessation of the treatment.

(6) The average numbers of corpora lutea of implantations were not significantly affected by treatment with Prometon Technical. The fetal body weight, mortality or sex ratio were not significantly altered either by the treatment of Prometon.

(7) No gross, soft tissue or skeletal variations noted in fetuses or pups were attributed clearly to maternal treatment with Prometon Technical under the test conditions described.

C. Discussions

(1) The preparation of Prometon Technical by Ciba-Geigy Corp. used in this study indicated that it was Batch FL NO.800793 with 80% active ingredients and 20% inerts. However, no information on the exact composition of the inerts was provided.

(2) Pregnancy occurred in 16, 13, 13, and 11 rabbits that received C (Vehicle), 0.5, 3.5 and 24.5 mg/kg/day dosages of Prometon Technical, respectively. The data represented pregnancy rates of 100%, 81%, 81%, and 69% in rabbits which were dosed 0, 0.5 3.5 and 24.5 mg/kg/day. Thus, the treatment of animals with Prometon Technical appeared to have had moderate effects on the pregnancy rates.

D. Conclusions

(1) Prometon Technical has moderate effects on the pregnancy rates in rabbits of presumed gestation at high dose such as 24.5 mg/kg/day.

(2) The anorexia and excess lacrimation, which occurred more frequently in rabbits receiving high doses, were attributed to a toxic effect of Prometon Technical.

(3) Prometon Technical appeared to have ^{teratogenic} no effect or very little ^{teratogenic} effect at low doses such as 0.5 and 3.5 mg/kg/day. "Minimum" rating can be considered only if and when the exact composition of the 20% inerts is provided and evaluated.

Core classification: Supplementary.

III. 90-Day Subchronic Feeding Study with Prometon Technical in Sprague-Dawley Rats (by William D. Johnson and Peter J. Becci of Food and Drug Research Labs., Inc., Waverly, NY. This study with FDRL Study No: 6805 was submitted to Ciba-Geigy Corp., Agricultural Division, Greensboro, N.C., on May 13, 1982).

A. Experimental

(1) The test chemical was Prometon Technical (Sponsor I.D.: Prometon Technical 98%, FL 801268, ARS 14; FDRL I.D.: 81-0019)

(2) Both male and female Sprague-Dawley rats were obtained from Charles River Breeding Labs., Inc., Wilmington, MA. Upon arrival and daily during the acclimation period (21 days), the rats were individually housed and had access to food (NIH open formula 07, certified feed, Zeigler Brothers, PA) and fresh tap water ad libitum.

(3) Animals were grouped by means of stratified randomization of body weights as in the following:

Group	No. of Animals		Dietary Level of Prometon Technical (ppm)
	Male	Female	
A-Control	35	35	0
B-Prometon	30	30	10
C-Prometon	30	30	50
D-Prometon	30	30	100
E-Prometon	35	35	300
F-Untreated	30	30	-

Each animal assigned to a group was given an established identification number. Animals in group F were used for pre-study clinical studies and were discarded prior to the start of feeding.

(4) The test diets were prepared by two steps. A premix containing Prometon Technical was prepared at first by adding the Prometon Technical directly to blank feed. The 300, 100, 50, 10 and 0 (vehicle as control) ppm diets were then prepared by the serious dilution of an appropriate aliquot of the premix with an appropriate amount of blank feed using a Hobart blender. The test diets were prepared bi-weekly for the first eight weeks of feeding and weekly for the last five weeks of feeding. The diet samples were taken from each dietary level each time a new batch of diet was prepared and chemically analyzed for the concentration of Prometon Technical prior to dosing.

(5) Animals in Groups B, C, D and E received the test chemical continuously in their diet for 90 consecutive days. Five rats per sex in the control and high dose groups were held for an

additional 28 days, as a recovery group. During this recovery period, the animals were fed basal diet without test chemical.

(6) The animals were observed daily for external signs of toxicity, including mortality checks, body weight and ophthalmological examination.

(7) The clinical studies, including hematology, clinical chemistry and urine analysis as listed in the report, were conducted accordingly.

(8) 20 animals per sex per group were sacrificed after 90 days of feeding and subjected to gross necropsies. The remaining 5 animals per sex in the control and high dose groups were sacrificed and subjected to gross necropsies after an additional 28 day recovery period.

(9) Fresh organ weights were obtained and organ-to-body weight and organ-to brain ratios were determined for the organs listed in the report.

(10) Paraffin embedded hematoxylin and eosin stained tissue sections, which were listed in the report, have been microscopically examined.

(11) The data were also analyzed statistically.

B. Results

(1) No animals died because of feeding diets with various concentrations of Prometon Technical.

(2) No daily clinical observations were seen that appeared any significant sign of toxicity or treatment-related behavioral abnormality.

(3) No significant differences in body weights between control and experimental groups were observed in both males and females during the feeding and recovery periods.

(4) A plot of food consumption versus time was made. A statistically significant ($p \leq 0.05$) increase in food consumption was observed in males at week 4 and in females at week 12 which were fed the test chemical at 300 ppm. However this increase in food consumption was not seen at other time periods, the increase in the particular time period for males or females can not be considered treatment-related.

(5) In the hematology study, the only statistically significant differences ($p \leq 0.05$) between any of the parameters occurred at termination in the high dose (300 ppm) males where an increase in the number of neutrophils and a decrease in the number of lymphocytes were seen in comparison to control values.

(6) In the study of clinical chemistry, globulin levels were seen significantly increased in females at 7 week-period and globulin levels were noted significantly decreased in the recovery females. However, these changes were transitory in nature, not dose dependent.

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- (7) A statistically significant ($p \leq 0.05$) increase in the mean absolute weight of the kidneys was seen in the high dose (300 ppm) females. The mean kidney-to-brain weight ratio was also significantly increased in the high dose females. No other differences between groups occurred with regards to absolute organ weights, organ-to-body weight ratio, or organ-to-brain weight ratios.
- (8) No significant gross or microscopic abnormalities were observed which might be considered as treatment-related.

C. Conclusions

- (1) The data from the feeding of Prometon Technical for 90 consecutive days to both male and female Sprague-Dawley rats at levels of 10, 50 and 100 ppm appeared no significant toxicological or histopathological effects.
- (2) However, the effects of Prometon Technical on the kidney weights and mean kidney-to-brain weight ratio in female rats at 300 ppm appeared attributable to the treatment. So the no-effect-level of Prometon Technical appeared to be 100 ppm, but not 300 ppm.
- (3) The test chemical Prometon Technical used in this study was 98% purity. No information on the other 2% whether inerts or impurities was provided. The answer for this question will be definitely needed.

Core classification: Supplementary. "Minimum" rating
can be considered only if and when the exact composition of the 2% inerts or impurities is provided and evaluated. This information is "MUST".

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