

8EHQ-0597-13944

AKIN, GUMP, STRAUSS, HAUER & FELD, L.L.P.

ATTORNEYS AT LAW

AUSTIN
BRUSSELS
DALLAS
HOUSTON
LONDON
MOSCOW
NEW YORK
PHILADELPHIA
SAN ANTONIO

A REGISTERED LIMITED LIABILITY PARTNERSHIP
INCLUDING PROFESSIONAL CORPORATIONS

1333 NEW HAMPSHIRE AVENUE, N.W.

SUITE 400

WASHINGTON, D.C. 20036

(202) 887-4000

FAX (202) 887-4288

WRITER'S DIRECT DIAL NUMBER (202) 887-_____



May 22, 1997

8EHQ-97-13944

Document Processing Center (TS-790)
(Attn: Section 8(e) Coordinator)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, D.C. 20460



88970000194

Contains No CBI

Re: TSCA Section 8(e) Reportable Information

Dear Sir or Madam:

Enclosed please find a completed TSCA Health & Safety Study Cover Sheet and audited draft report derived from a developmental toxicity study, conducted on behalf of the fragrance materials industry, entitled "Oral (Gavage) Developmental Toxicity Study of 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-Hexamethyl Cyclopenta-Gamma-2-Benzopyran (HHCB) in Rats." This submission is made in accordance with reporting responsibilities under Section 8(e) of the Toxic Substances Control Act, and is still under review by the sponsor. This submission is made within the fifteen day reporting time-frame provided by the statute. 15 U.S.C. § 2607(e).

The study reported no deaths, abortions, or premature deliveries. The test article, HHCB, was not selectively toxic to development based on the study results, as it showed a maternal NOAEL of 50 mg/kg/day and a developmental NOAEL of 150 mg/kg/day. However, at a dosage of 500 mg/kg/day, statistically significant ($p \leq 0.01$) increases in the incidence of axial skeletal (vertebral/rib) fetal malformations, as compared to the concurrent control group incidences and historical experience, were observed. These findings were considered to be associated with administration of this dosage of the test article to the female rats based on the dosage dependent pattern of the effect. Statistically significant reduced fetal body weights and increased incidences of fetal variations and malformations were also reported for the 500 mg/kg/day dosage group.

May 22, 1997

Page 2

Consistent with EPA's current interpretation of Section 8(e), the observed results would appear to be reportable; however, we believe that these findings do not present any real indication of potential for "substantial risk of injury to health."

The potential health impact of the development defects reported at a dosage of 500 mg/kg/day in rats is negligible when assessed in relation to expected human exposure. HHCB is used as one of many fragrances in consumer products. Its characteristics of high molecular weight and low volatility would indicate that the potential of exposure due to inhalation is low.

Studies on dermal exposure are informative in placing potential risks in perspective. The estimate of dermal exposure involving cosmetic products provides a skin exposure of 0.76 mg/kg/day and an absorbed systemic exposure of 0.11 mg/kg/day which provides a margin of 1364-fold over the exposure that had no effect on developmental toxicity. In addition, a 13-week oral dietary admixture toxicity study provided an NOAEL of 150 mg/kg/day.

Should you have questions or concerns regarding this submission, please feel free to contact me.

Sincerely,


Roger J. Marzulla

Enclosures

MB/fj

TITLE: ORAL (GAVAGE) DEVELOPMENTAL TOXICITY STUDY OF
1,3,4,6,7,8-HEXAHYDRO-4,6,6,7,8,8-HEXAMETHYL
CYCLOPENTA-GAMMA-2-BENZOPYRAN (HHCB) IN RATS

ARGUS RESEARCH LABORATORIES, INC.
PROTOCOL NUMBER: 1318-001

I. SUMMARY AND CONCLUSION

A. Methods^a

Twenty-five CrI:CD®BR VAF/Plus® (Sprague-Dawley) presumed pregnant female rats were assigned to each of four dosage group (Groups I through IV). The test article, 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-Hexamethyl Cyclopenta-gamma-2-Benzopyran (HHCB), or vehicle, Mazola® Corn Oil, was administered orally (via gavage) once daily to female rats on day 7 through 17 of presumed gestation (DGs 7 through 17). Dosages of 0 (Vehicle), 50, 150 and 500 mg/kg/day of the test article were administered at a dosage volume of 5 mL/kg, adjusted daily on the basis of the individual body weights recorded before intubation. The rats were intubated once daily at approximately the same time each day.

The female rats were observed for viability at least twice each day of the study. The rats were also examined for clinical observations of effects of the test article, abortions, premature deliveries and deaths before and approximately one hour after dosage. These observations were also conducted once daily during the postdosage period (DGs 18 through 20). Body weights were recorded on DG 0 and daily during the dosage and postdosage periods. Feed consumption values were recorded on DGs 0, 7, 10, 12, 15, 18 and 20.

All rats were sacrificed by carbon dioxide asphyxiation on DG 20, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions.

Each fetus was weighed and examined for sex and gross external alterations.

-
- a. Detailed descriptions of all procedures used in the conduct of this study are provided in the appropriate sections of this report and in APPENDIX C (PROTOCOL AND AMENDMENT).

Approximately one-half of the fetuses in each litter were examined for soft tissue alterations. The remaining fetuses in each litter were eviscerated and examined for skeletal alterations.

B. Results

No deaths, abortions, or premature deliveries occurred during the study. All rats survived until scheduled sacrifice.

The 500 mg/kg/day dosage group had four to nine ($p \leq 0.01$) rats with excess salivation, urine-stained abdominal fur, red or brown substance on the forepaws and alopecia. All necropsy observations were considered unrelated to the test article.

Body weight gains were unaffected by the 50 mg/kg/day dosage of the test article. The 150 and 500 mg/kg/day dosage groups had statistically significant ($p \leq 0.05$ to $p \leq 0.01$) dosage-dependent reductions in maternal body weight gains for the entire dosage period (calculated as DGs 7 to 18). These effects of the test article reflected significantly reduced ($p \leq 0.01$) weight gain in the 150 mg/kg/day dosage group and significant weight loss ($p \leq 0.01$) in the 500 mg/kg/day dosage groups on DGs 7 to 10. Weight gains in the 150 mg/kg/day dosage group were comparable to the control group values for the remainder of the dosage period and the post-dosage period. The initial weight loss in the 500 mg/kg/day dosage group was followed by a significant increase ($p \leq 0.01$) in weight gain on DGs 10 to 12, a rebound phenomenon commonly observed in these types of studies, after which weight gains were comparable to control group values. As the result of these effects, maternal body weight gains for the entire interval after initiation of treatment (DGs 7 to 20) were reduced and significantly reduced ($p \leq 0.01$) in the 150 and 500 mg/kg/day dosage groups, respectively.

Dosage-dependent, significant reductions ($p \leq 0.01$) in maternal body weights were generally evident in the 500 mg/kg/day dosage group on DGs 8 through 20. All other significant differences ($p \leq 0.05$ to $p \leq 0.01$) in maternal body weight averages reflected the significantly lower ($p \leq 0.01$) DG 0 body weights in the three groups administered the test article, as compared with the control group value, and were the result of study design constraints that resulted in separate randomizations of the control group and two groups of 175 rats, of which 75 were randomly assigned to this study, and 75 to a companion study (1318-002) with 25 rats assigned to the control group, in an effort to conserve animal use.

Absolute and relative feed consumption values were unaffected by the 50 mg/kg/day dosage of the test article. Absolute (g/day) and relative (g/kg/day) feed consumption values for the entire dosage period (calculated as DGs 7 to 18) were reduced ($p \leq 0.05$ to $p \leq 0.01$) in the 150 and 500 mg/kg/day dosage groups, reflecting significant reductions ($p \leq 0.05$ to $p \leq 0.01$) in absolute feed consumption values on DGs 10 to 15 in the 150 mg/kg/day dosage group and in absolute and relative feed consumption values in the 500 mg/kg/day dosage group on DGs 7 to 12, and in the absolute feed consumption value on DGs 12 to 15. After completion of the dosage period, the 500 mg/kg/day dosage group had a significantly increased ($p \leq 0.01$) relative feed consumption value on DGs 18 to 20, a rebound phenomenon commonly observed in these types of studies. These effects of the test article resulted in significant reductions ($p \leq 0.05$ to $p \leq 0.01$) in absolute and/or relative feed consumption values in the 150 and 500 mg/kg/day dosage groups for the entire interval after initiation of dosage (DGs 7 to 20) and in the 500 mg/kg/day dosage group for the entire gestation period (DGs 0 to 20).

Pregnancy occurred in 21 to 25 of the 25 female rats in each dosage group. There were 25, 24, 24 and 21 pregnant dams Caesarean-sectioned on DG 20 in the 0 (Vehicle), 50, 150 and 500 mg/kg/day dosage groups, respectively.

The 500 mg/kg/day dosage group had significantly reduced ($p \leq 0.01$) fetal body weights, as compared with the control group values. This observation was considered an effect of the test article because it occurred in the high dosage group, was statistically significant, and the values were clearly lower than those for any other dosage group, despite the values being within ranges observed historically at the Testing Facility. No other Caesarean-sectioning and litter parameters were affected by dosages of the test article as high as 500 mg/kg/day. There were no other dosage-dependent or statistically significant differences in the litter averages for corpora lutea, implantations, litter size, live/dead fetuses, early/late resorptions, percent resorbed conceptuses, or percent male fetuses. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses.

Malformations occurred in one, zero, two and six ($p \leq 0.01$) fetuses from one, zero, two and five ($p \leq 0.01$) litters in the four respective dosage groups. The significant increases in the litter and fetal incidences of malformations in the 500 mg/kg/day dosage groups were considered to be associated with administration of this dosage of the test article to the dams based on the dosage-dependent pattern of effect [statistically significant ($p \leq 0.01$) increases in the incidences of axial skeleton (vertebral/rib) malformations, as compared with the concurrent control group incidences and the historical experience of the Testing Facility]. Malformations occurring in fetuses of dams administered dosages less

than 500 mg/kg/day were considered unrelated to the test article because the severity of the expression of the malformation was not consistently dosage-dependent and in some fetuses, malformations of concern were present with other alterations considered sporadic in nature because of the absence of an embryologically-based association. All other gross external, soft tissue and skeletal malformations or variations in the fetuses were considered to be unrelated to the test article.

Ossification of the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum, forelimbs and hindlimbs occurred at similar incidences in litters in all dosage groups. Analyses of the average numbers of fetal ossification sites per litter did not reveal biologically important or statistically significant differences among the four dosage groups, with the exception of the significantly decreased ($p < 0.05$) number of ossification sites in the metatarsals of the 500 mg/kg/day dosage group. The decreased number of metatarsal ossification sites observed was considered unrelated to the test article because it was within historical control ranges.

C. Conclusion

The maternal no-observable-adverse-effect-level (NOAEL) for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-Hexamethyl Cyclopenta-gamma-2-Benzopyran (HHCB) is 50 mg/kg/day. Maternal toxicity was evident in the 150 and 500 mg/kg/day dosage groups as adverse clinical observations and significant reductions in body weight gains and feed consumption values. The developmental NOAEL for the test article is 150 mg/kg/day. The 500 mg/kg/day dosage was associated with increased incidences of fetal skeletal variations, reduced fetal body weights and increased incidences of fetal variations and malformations. Based on these data, the test article was not selectively toxic to development because adverse effects on development occurred only at dosages which showed toxic effects (adverse clinical observations, decreased body weight and feed consumption values) in the dams.

Mildred S. Christian, Ph.D., ATS Date
Executive Director of Research

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

Robert M. Parker, Ph.D., DABT Date
Senior Scientist and Study Director

Best Available Copy