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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: 04 NOV 1980

SUBJECT: Methoprene [Isopropyl-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate]
Cuewell No. 28 AAA

FROM: G.Z. Ghali, Ph.D.
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TO: Mr. Franklin Gee, PM #17
Registration Division (TS-767)

THRU: William Burnam, Acting Branch Chief
Toxicology Branch, HED (TS-769)

Action Requested:

Review of rabbit and mouse teratology studies submitted by Zoecon Corporation to support registration of Methoprene (Altosid, technical Acc # 242045).

Conclusions and Recommendations:

I. Rabbit Teratology:

This study does not show evidence of teratogenicity. However, this study is lacking a positive control to indicate the sensitivity of this particular strain of Japanese rabbits to any chemical teratogen.

From the current study the no effect level (NEL) is considered to be 200 mg/kg/day, and the least effect level (LEL) is considered to be 2000 mg/kg/day for embryo lethality in utero.

This study is classified as Core minimum. The registrant is being requested to submit historical terata incidence for this strain of rabbit.

II. Mouse Teratology:

This study is classified as supplementary. The following additional data are required inorder to allow proper review of the study for teratogenic and fetotoxic potential.

1. Absolute and relative internal organs weight for individual animals (tabulated).

2. Data on ossification and detail descriptions of vertebrae for individual animals (tabulated).

3. Toxicology Branch requires a statement from the registrant or testing laboratory addressing the possibility of a bimodal effect (or an effect at lower dose level) on the maturation of males and females as it is manifested by the delay in the descent of testes and the slowing of the opening of the vagina.

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EPA Form 1320-6 (Rev. 3-76)

I. Rabbit Teratology:

Review and Method:

This study (NRI-PL-74-2405) dated July 1975 was conducted by Nomura Research Institute (Japan) on four groups of nine-month old nulliparous Japanese rabbits, acclimated for one month prior to study. Each group consisted of 10 pregnant animals. A preliminary subacute toxicity study was done for dose range determination. Dosage levels of 0, 50, 200 or 2000 mg/kg of the technical grade (95.7% a. i.) were administered daily in olive oil by gavage to pregnant rabbits from day 7 to day 18 of pregnancy. General symptoms were observed daily from day 0 to day 28 of pregnancy. Weight was measured every two days. On day 28 of pregnancy, fetuses were delivered by Caesarean sections and examined. Fetal distribution, number of implantation sites and mortality were recorded. Mortality was divided into four categories, i.e. resorption site, early, mid or late fetal death. Weight, height and tail length of the viable fetuses were measured. Sex was recorded and abnormalities of external morphology, including oral cavity, and internal organs were noted. Skeletal preparations were made by Dawson's method and examined for any malformations.

Results and Discussion:

1. Influence on pregnant animals:

A significant reduction (33.4%) of body weight gain of pregnant rabbits was observed at 2000 mg/kg indicating maternal toxicity.

Two cases of abortion occured at 2000 mg/kg also indicating maternal toxicity of the compound at this dose level.

There were no abnormalities in general symptoms and behavior of mothers during pregnancy.

2. Number of implantations and fetal mortality:

No significant effect on the number of implantations was observed when treated were compared to controls in the treated animals compared to control. Dead embryos increased from 5% in the control to 20.5% at the highest dose level (2000 mg/kg/day), while at 50 mg/kg embryonic death accounted for 12.4% and at 200 mg/kg accounted for 7.7% loss. The effect therefore is not considered to be dose related.

3. Number of fetuses per litter:

The average number of live fetuses was not significantly effected i.e. 7.9 ± 1.9, 7.8 ± 2.9, 7.2 ± 2.0 and 7.5 ± 2.6 for the control, 50, 200, and 2000 mg/kg respectively.

4. Weight, Length and sex of live fetuses:

The average body weight of fetuses was not affected i.e. 33.8 ± 5.7, 38.4 ± 7.7, 35.0 ± 7.0 and 39.7 ± 5.9 for males, and 37.6 ± 5.5, 38.4 ± 7.7, 35.0 ± 7.0 and 35.8 ± 8.9 for females in 0, 50, 200, 2000, mg/kg/day group respectively.

No significant effect on body length of fetuses (crown to rump), but a slight effect on the tail length was observed at 200 and 2000 mg/kg/day.

The sex ratios (F/M) were 1.14, 1.05, 1.40 and 1.60 for 0, 50, 200 and 2000 mg/kg/day groups respectively.

5. External malformations:

No external malformations were observed.

6. Visceral malformations:

Only one case of abnormal kidney position was observed in a male of 200 mg/kg/day group. It seems to be an incidental case since it was not observed in other groups including 2000 mg/kg/day.

7. Skeletal malformation:

Incidence of variations in the number of ribs in the treated animals were comparable to those of the control.

The incidence of delayed fusion of sternal ossification centers was 24.1% in control, 19.2% at 50 mg/kg, 19.4% at 200 mg/kg and 5% at 2000 mg/kg.

Conclusion:

This study does not show evidence of teratogenicity. However, this study is lacking a positive control to indicate the sensitivity of this particular strain of Japanese rabbits to any chemical teratogen.

From the current study the no effect level is considered to be 200 mg/kg/day, and the LEL is considered to be 2000 mg/kg for embryo lethality in utero.

This study is classified as Core minimum . The registrant is being requested to submit historical terata incidence for this strain of rabbit.

II. MOUSE TERATOLOGY

Review and Method:

This study (NRI-PL-74-2465) dated October 1975 was conducted by Nomura Research Institute (Japan) on four groups of 10 week old ICR lineage mice acclimated for one week prior to the study. A preliminary subacute toxicity study was done for dose selection. Consequently, the dosage levels of 0, 50, 200 and 600 mg/kg were chosen for the teratology study. Methoprene (Altosid) technical grade (95.7% a.i. lot# PC 060054 run 55) in olive oil was orally administered on daily basis from day 7 to day 14 of pregnancy. At least 30 mice were used for each dose level. Weight of pregnant mice was monitered three times a week. Food and water consumptions were measured every four days.

On the 18th day of pregnancy, 20-23 mice of each group were anesthetized with ether and exsanguinated. The uterus was opened and Fetal number, sex and mortality were recorded. The fetuses were examined for external and visceral abnormalities. Then by Dawson's (1962) method, skeletal preparations were made and examined for malformations.

10-14 pregnant mice from each group were allowed to whelp naturally. For 3 weeks, the neonates were reared by the mother. During this period, general symptoms of mothers and pups were observed daily. Also during this period for 3 times a week, the average weight of each litter was measured. The following developmental parameters were recorded: ear development, hair growth, and opening of eye lids. The internal organs of the mother were inspected at necropsy the 21st day following parturition. The number of implantation scars was also recorded. Weanling mice from 5 litters in each group were inspected for behavioral change, and external abnormalities at 21 days. At necropsy, after gross inspection for abnormalities in internal organs, the following organs were weighed: heart, lungs, liver, spleen, kidneys, and testes. Skeletal preparations were made by Softex and examined for malformation. The remaining weanling infants were separated by sex, and maintained 7 weeks. During this period, each mouse was weighed weekly, and as an index of maturity, time periods for descent of the testes and opening of the vagina were determined. At 70 days of age, the animals were inspected for abnormalities in behavior and external morphology. After inspection of internal organs the following organs were weighed: heart, lungs, liver, spleen, kidneys, and testes. Also according to standard procedures, H&E stained slides of testes and ovaries were prepared for histological examination. Examination for skeletal abnormalities was done with Softex.

Results and Discussion:

Effect on dam:

1. Maternal mortality and behavioral changes: no maternal mortality or behavioral changes were observed in the treated females.

2. Maternal body weight gain and food and water consumptions: A significant increase over control in the rate of body weight gain during pregnancy was observed in the treated females (P=0.1%).

   Some significant increase in the rate of water consumption was reported at the lowest dose level (50 mg/kg) in the first five days of pregnancy. A significant decrease in food consumption was also observed at the fifth day of pregnancy in the 200 mg/kg group. However, neither of these effects was a compound related effect and are considered to be of little toxic significance in this case.

3. Gestation period: No effect on the duration of gestation period was observed. The average duration of gestation ranged from 18.6-19.0 days in all groups.

Effect on fetuses:

1. Number of implantations and dead fetuses: The average number of implantations has been increased in a dose related manner. This average was 11.3, 11.7, 12.2 and 12.9 for the control, 50, 200 and 600 mg/kg group respectively. The number of mortalities also decreased in the treated animals, also in a dose dependent manner. Percent mortalities were 17%, 16.2%, 12.2% and 11.2% for the control, 50, 200 and 600 mg/kg groups.

2. Fetus body weight: The average fetus body weight was slightly increased i.e. 1.34 $\pm$.38, 1.38 $\pm$.16, 1.40 $\pm$ .13 and 1.39 $\pm$.16 g. for males and 1.27 $\pm$.14, 1.32 $\pm$.16, 1.37 $\pm$.15 and 1.33 $\pm$ .12 for females respectively in the control, 50, 200 and 600 mg/kg groups.

3. External abnormalities: No external abnormalities were observed in either control or treated groups.

4. Visceral abnormalities: No visceral abnoralities were observed in any group in the study.

5. Skeletal abnormalities: No major skeletal malformations were seen either in the control or in the treated groups.

The incidence of 14 ribs, unilaterally or bilaterally, was 50.0%, 52.0%, 47.9% and 44.1% in the control, 50, 200 and 600 mg/kg groups respectively.

The caudal vertebrae were 7.13, 8.57 and 8.40 in the control, 50, 200 and 600 mg/kg groups respectively.

Effect on weanlings:

The ratio of the total number of viable fetuses to implantation sites was 88.9%, 92.7%, 90.8% and 82.7% respectively in the control, 50, 200 and 600 mg/kg groups. The ratio of neonates surviving after 3 weeks to live births was 95.8%, 71.1%, 87.8% and 94.5% in the control, 50, 200, 600 mg/kg groups. In the 50 mg/kg group the ratio of viable fetuses to total implantation sites is high (88.9%), but percentage of reared infants was the lowest observed (71.1%).

Effect on neonatal growth:

Although there was a considerable difference in weight among groups, after 4 weeks, males and females gained weight normally.

The time of external ear development, hair growth, opening of lids, descent of testes, and opening of the vagina were measured as function of growth and maturity. In the 200 mg/kg group the descent of testes was retarded several days compared to control (25.6 vs 27.5 days) and in the 50 mg/kg group the opening of the vagina was similarly retarded several days compared to control (30.0 vs. 32.6 days).

a. At three weeks

In the five litter that were necropsied at 3 weeks of age, no change in general behavior and no abnormalities in major organs were observed. There was no skeletal malformations seen with Softex examination.

The weight of the heart the decreased in the 50 mg/kg females (p=1%).

The weight of the lungs increased in the 600 mg/kg females and males (p=5%).

The weights of liver (p=1%) and kidneys (P=5%) were increased in 600 mg/kg males.

The weight of the spleen decreased in the 50 mg/kg females and the 200 mg/kg males.

In all treated groups (males and females) relative weight of the spleen decreased.

In all treated groups the relative weight of testes decreased with statistical significance (p=1%).

b. At ten weeks

The remaining mice from 5-9 litters were necropsied at ten weeks of age. No behavioral, visceral, and skeletal (Softex) abnormalities were seen.

The weight of the heart increased in the 50 and 200 mg/kg females at P=1% and P= 5% respectively.

The weight of lung in the 50 mg/kg group males decreased (P=5%).

The weight of the spleen in the 50 mg/kg females increased (P=1%).

The weight of the kidneys of females in all treated groups increased (P=1%).

The testicular weight at 50 and 200 mg/kg groups decreased (P=1%).

The relative weight of heart in the 200 mg/kg females increased (P=5%).

The relative weight of lungs in the 50 mg/kg (P=1%) males and 600 mg/kg (P=5%) females decreased.

The relative weight of the spleen in the 50 mg/kg females increased (P=1%).

The relative weight of kidney in 50 and 200 mg/kg females increased (P=1%).

The relative weight of testes at 50 and 200 mg/kg decreased (P=1%).

Histological examination of ovaries and testes in the 50 mg/kg group revealed one example of atrophy of seminiferous tubules.

Conclusion:

This study is classified as supplementary. The following additional data are required inorder to allow proper review of the study for teratogenic and fetotoxic potential.

1. Absolute and relative internal organs weight for individual animals (tabulated).

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