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CASWELL FILE

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

SUBJECT: EPA Reg. #279-2712; 279-2876; Carbofuran Teratogenic Studies
CASWELL #160A Accession#244388-90

FROM: William Dykstra, Toxicologist
Toxicology Branch, HED (TS-769) WDD 7DC 3/17/81

TO: Jay Ellenberger (12)
Registration Division (TS-767) WJB

Recommendations:

- 1) Carbofuran is not teratogenic in the rat at dietary levels up to 160 ppm during gestation days 6-19. The NOEL for fetotoxicity in the postnatal period is considered to be 20 ppm. The study is acceptable as core-minimum data.
- 2) Carbofuran is not teratogenic in the rat at gavage doses up to 1.2 mg/kg/day during gestation days 6-15. The NOEL for fetotoxicity is also 1.2 mg/kg/day. The study is acceptable as core-minimum data.

Review:

- 1) Pilot Teratology Study in Rats with Carbofuran in the Diet (IRDC Report#167-153; December 26, 1980)

Groups of ten pregnant Charles River COBS CD rats were used to determine dose levels of Carbofuran for a teratology study. Carbofuran was dissolved in ACS grade acetone and dose levels of 0, 20, 60, 120, 160 and 200 ppm were administered orally by dietary inclusion on gestation days 6 through 19. The control group received the basal laboratory diet, Purina Certified Laboratory Chow #5002 with an equivalent amount of ACS grade acetone, on a comparable regimen. Uterine examinations were performed on gestation day 20.

Results:

Survival in all carbofuran treated groups was 100%. There were no biologically meaningful differences in mean body weight gain, or in mean uterine examination observations in any of the treatment groups when compared to the control group.

Dried red matter in the nasal region, soft stool, matting of the haircoat and scabbing were observed infrequently in the carbofuran treated groups. Dose-related mean maternal body weight losses and decreases in mean food consumption were noted during the first two days of test diet administration in the 60, 120, 160 and 200 ppm treatment groups. Over the entire treatment period, mean body weight in the 60 ppm group was comparable to the control group, was moderately decreased in the 120 ppm group and severely decreased in the 160 and 200 ppm groups. Mean food consumption in the 60 ppm group over the entire treatment period was comparable to the control group and was moderately decreased in the 120, 160 and 200 ppm dosage groups.

Conclusion:

Based on the results of this study, dose levels of 0, 20, 60 and 160 ppm were selected for a teratology study in the rat with carbofuran in the diet.

Classification: Supplementary Data

(a) Pilot Study

2) Teratology and Postnatal Study in the Rat with Carbofuran (IRDC Report#167-154; January 8, 1981)

One hundred and sixty untreated, sexually mature, virgin female Charles River COBS CD rats were used to determine the potential teratogenic and postnatal effects of carbofuran. One male and one female rat of the same strain and source were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug or by a vaginal smear for sperm. The day that evidence of mating was detected was designated day 0 of gestation and the female was returned to an individual cage. Females scheduled to deliver (for postnatal evaluation) were placed in plastic cages with ground corn-cob bedding prior to parturition and throughout lactation.

Mated females were consecutively assigned according to random placement in a block design to a control group (0 ppm) and three treatment groups (20, 60, 160 ppm) consisting of 40 rats each and each group was administered the appropriate dosage orally by dietary administration on gestation days 6 through 19.

Prior to treatment, the females were observed daily for mortality and overt changes in appearance and behavior. Females were observed daily for clinical signs of toxicity and twice daily for viability on day 6 of gestation through day 21 of lactation. Dams not surviving to the scheduled sacrifice date were necropsied in an attempt to determine the cause of death. Individual female body weights were recorded on the corresponding gestation body weight days and calculated for the following intervals: 0-5, 6-7, 8-9, 10-11, 12-13, 14-15, 16-17 and 18-19.

On day 20 of gestation, a sufficient number of females (the first 20 to 23 from each group) were randomly selected to ensure that 20 pregnant females were sacrificed from each group. The females were sacrificed by carbon dioxide inhalation. Immediately following sacrifice, the abdominal cavity was opened to expose the uterus and ovaries. The uterus was excised and weighed prior to removal of the fetuses. The number and location of viable and nonviable fetuses, early and late resorptions and the total number of implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological and pathological changes and the carcasses discarded. Maternal tissues were preserved in 10% neutral buffered formalin for microscopic examination only as deemed necessary for gross findings. Uteri from females that appeared nongravid were opened and placed in an approximate 10% ammonium sulfide solution for confirmation of pregnancy status.

The remaining females from each group were allowed to deliver.

All fetuses were individually weighed, measured for crown-rump length and examined for external malformations and variations, including the palate and eyes. Each fetus was externally sexed and individually numbered and tagged for identification. Each fetus was dissected, internally sexed and examined for visceral malformations and variations. The heart was dissected by a modification of the method described by R.E. Staples. The heads of approximately one-half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning as described by Wilson. All fetuses (including those with the heads removed) were eviscerated, fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson for subsequent skeletal examination.

The remaining females from each group were placed in plastic breeding cages with ground corn-cob bedding. During the period of expected parturition, the F_0 females were observed twice daily for newborn litters. Lactation day 0 was designated as the day the entire litter was found and judged complete. Any difficulties occurring at parturition were recorded. On lactation day 0, the litters were sexed and examined for litter size, stillbirths, live births and any gross anomalies. The dams and pups were individually weighed on lactation days 0, 7, 14 and 21. Pups were weighed additionally on lactation day 4. The dams and litters were observed daily for overt signs of toxicity, changes in appearance and behavior and mortality.

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The presence of dead pups was recorded and all intact dead pups dying during lactation were examined for visceral and skeletal anomalies.

On the 25th day after termination of mating, a gross necropsy was performed on all females where evidence of mating was found but which failed to deliver and any condition which would have prevented pregnancy was recorded.

On lactation day 21, the remaining dams and all pups were sacrificed by carbon dioxide inhalation. The dams were necropsied and grossly examined for remarkable morphological and pathological changes. The pups were examined for external malformations and variations, including the palate and eyes. Each pup was dissected, internally sexed and examined for visceral malformations and variations, including the brain by a mid-coronal slice. The heart was dissected by a modification of the method described by R.E. Staples. The kidneys from 10 randomly selected pups from each dose group were preserved in 10% neutral buffered formalin for possible future histopathological examination. The carcasses of all dams and pups were discarded.

Statistical analyses of the data were performed.

Results:

Survival was 100% in the dams of control and all carbofuran treated groups. No biologically meaningful differences in behavior were observed in any of the rats in the treated groups when compared to the control group. Hair loss, primarily on the limbs, was observed in all groups, including the control, during gestation and lactation; a slight increase was observed in the treated groups although not in a dose-related pattern. Soft stool was observed with similar frequency in all groups, including the control, during the treatment period, although in several of the treated rats, an increase in the duration of the condition was noted when compared to the control group. During the lactation period, soft stool was observed infrequently in the treated groups. An occasional instance of scabbing, primarily on the limbs, was noted in all groups during treatment and in a few rats in the 60 and 160 ppm groups during lactation. Matting, primarily of the ventral haircoat, was observed in a few rats in the 60 and 160 ppm groups during treatment and lactation. Red matter, either in the nasal or anogenital region, was observed in a few animals only in the 60 ppm group during gestation.

At cesarean section, hydrometra was observed in one female in each group. Dam#'s 63641, 63678, 63715 and 63742 in the control, 20, 60 and 160 ppm groups, respectively. Pitted kidneys were noted in one dam in the control group (#63644); hydronephrosis of one kidney was noted in one dam in the 160 ppm group (#63758). The spleen of one dam each in the control (#63625) and 160 ppm group (#63748) was enlarged, adhered to the abdominal fat, liver and/or pancreas and contained numerous yellow foci. Histopathological examination revealed the presence of multifocal pyogranulomata in the spleen of dam #63625

and focal capsular fibrosis in the spleen of dam #63748. A subcutaneous mass palpated in the right axillary region of a female in the 60 ppm group (#63709) was found to be a mammary gland adenocarcinoma upon histological examination.

Four females did not deliver, one each from the 20 and 60 ppm groups and two from the 160 ppm group; all were found to be nongravid and three of the four females were internally normal. Hydrometra was noted in the 20 ppm group female. At the scheduled sacrifice on lactation day 21, hydrometra was noted in two females, one each from the control (#63646) and the 20 ppm group (#63694). All remaining dams were internally normal.

There were no statistically significant decreases in mean maternal body weight gains in the 20 ppm group when compared to the control group over the period of gestation (gestation days 6 to 19). Statistically significant dose-related mean maternal body weight losses occurred in the 60 and 160 ppm groups during the first two days of treatment. These body weight losses, as well as statistically significant decreases in mean body weight gain during gestation days 8 to 10 (in the 160 ppm group), days 18 to 20 (in the 60 and 160 ppm groups) contributed to a significantly reduced mean body weight gain in the 60 and 160 ppm groups over the entire treatment and gestation intervals. The mean adjusted body weight gain was also significantly reduced.

Decreases in mean maternal body weight gain were noted in the 60 and 160 ppm groups during the first week of lactation (days 0 to 7) when compared to the control group. The decrease in the 160 ppm group was statistically significant. Slight increases although not statistically significant, in mean body weight gain were noted in these groups between lactation day 7 to 14. Over the entire lactation period (days 0 to 21) mean body weight gains in all of the treated groups were comparable to the control group.

There were no statistically significant difference in the mean food consumption in the 20 ppm group when compared to the control group during days 6 to 18 gestation. A statistically significant decrease in this test group was noted during gestation days 18 through 19. However, this slight reduction was not considered biologically significant being less than 10%. A statistically significant decrease occurred in mean food consumption in the 60 and 160 ppm groups during gestation days 8 through 9. In addition in the 160 ppm group, a significant decrease was noted during gestation days 10 through 11. Statistically significant increases occurred in the 60 and 160 ppm groups at various intervals from gestation days 14 through 19. Evaluated statistically over the entire treatment period, mean food consumption in the 20 and 60 ppm treatment groups was relatively comparable to the control groups and mean food consumption in the 160 ppm group was slightly reduced.

There were no statistically significant differences in the numbers of corpora lutea, total implantations, early or late resorptions, mean fetal crown-rump length, mean fetal body weight or fetal sex distribution in any of the treated groups when compared to the control group.

There were no statistically significant differences in the number of litters with malformations in any of the treatment groups when compared to the control group. The number of fetuses with malformations, and developmental or genetic variations observed in the treated groups were comparable to the control group.

There were no difficulties observed at parturition in any group. The mean length of gestation in each treated group was comparable to the control group. The fertility index in each treated group was slightly less than the control however, decreases are not indicative of a compound effect as implantation occurred prior to administration of carbofuran. The mean number of viable pups per litter at lactation day 0 and survival indices at all measurement intervals in each of the treated groups were comparable to the control group. There were no biologically meaningful differences in the appearance or behavior of pups in the treated groups when compared to the control group. Mean pup body weights in the 20 ppm group were very slightly decreased on a few of the measurement days, however, these were not considered to be biologically meaningful. Slight, although not statistically significant, decreases in mean pup body weights were noted in the 60 ppm group on lactation days 0, 4, 7, 14, and 21 (male and female). In the 160 ppm group, statistically significant decreases in mean pup body weights occurred on lactation days 0, 4, 7, 14 and 21 (male and female).

Seven intact dead pups were necropsied and examined skeletally. Four of the seven pups were internally normal (one pup per litter from control (Dam#63661), 20 ppm (Dam#63701, #63686) and 60 ppm (Dam#63725)). Skeletal variations were observed in three pups; bilateral rudimentary ribs in two pups (one pup per litter from Dam#63725 (60 ppm) and #63652 (control)) and a right rudimentary rib in another (one pup from Dam#63725 (60 ppm)).

At the scheduled sacrifice on lactation day 21, hydronephrosis was observed in three male pups, two in the 60 ppm group (Dam#'s 63722 and 63724) and one in the 160 ppm group (Dam#63769). In addition, both testes of one male pup (Dam#63777) in the 160 ppm group were approximately one-third the normal size.

Conclusion:

Carbofuran was not teratogenic when administered in the diet at levels up to 160 ppm during gestation. The fetotoxic NOEL is considered to be 20 ppm. The LEL is 60 ppm and the effect was reduced body weight in pups of both sexes in the postnatal phase of the study.

Classification: Core-Minimum Data

3) Teratology Study in the Rat with Carbofuran (IRDC Report#167-155; December 26, 1980)

Groups of 25 pregnant Charles River COBS CD rats were treated daily by gavage with carbofuran at doses of 0.25, 0.50 and 1.20 mg/kg on days 6 through 15 of gestation. A control group received the vehicle, corn oil, on a comparable regimen. Cesarean sections were performed on all females on gestation day 20.

Results:

Survival was 100% in all dosage groups. There were no statistically significant differences in the mean numbers of corpora lutea, total implantations, early and late resorptions, post-implantation loss, viable fetuses, the fetal sex distribution, mean fetal body weight or the number of litters with malformations in any of the carbofuran treated groups when compared to the control group. Mean fetal body length and the number of litters and fetuses with malformations and genetic and developmental variations were also comparable to the control group. However, slight increase in non-dose related developmental variations occurred at the mid-dose of 0.50 mg/kg/day. They consisted of, for example, sternebrae #5 and/or #6 unossified; and 7th cervical rib.

Conclusion:

Carbofuran is not teratogenic at gavage doses up to 1.2 mg/kg/day during gestation. The fetotoxic NOEL is also 1.2 mg/kg/day

Classification: Core-Minimum Data

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