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CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carbofuran Toxicity Data
CASWELL#160A Accession#099276-283

FROM: William Dykstra
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1) Two-Year Dietary Toxicity and Carcinogenicity Study in Rats (CIRDC Report#167-115, Dec. 18, 1979)

Test Material: Carbofuran, tech. 95.6%; Furadan Insecticide; MrS1314; 5 lbs, 3/16/77, brown fibrous powder with dark brown or black chunks.

Three hundred sixty male and 360 female weanling Charles River CD rats obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass., and weighing from 76 to 143 grams and 68 to 134 grams, respectively, were initiated in this study. The animals were placed inot four groups (one control and three treated) as indicated below:

Group	Number of Animals		Dosage Level (ppm))
	Male	Female	
1 (Control)	90	90	0
2	90	90	10
3	90	90	20
4	90	90	100

Five additional rats per sex per group were initiated in the study as possible replacement animals. Rat 55554, group III female replaced rat 55291 which died week 4. Rat 55553 group IV female replaced rat 55471 which died week 1 of study. All of the replacement rats were sacrificed and discarded at the end of 4 week replacement period. The rats were housed individually in suspended wire-mesh cages and maintained in a temperature-, humidity-, and light-controlled room. Water and the control and test diets were available ad libitum. The study was initiated on May, 20, 1977. All surviving animals were sacrificed and necropsied between May 18 and 31, 1979.

The rats were observed twice daily for signs of overt toxicity and mortality. Detailed observations, including descriptions of palpable masses were recorded weekly. Individual body weights were measured weekly. Individual food consumption was calculated weekly.

Ophthalmoscopic examinations were performed for all rats once during the pretest period and at 12 and 24 months of study.

Hematologic and biochemical studies (including blood and brain cholinesterase activity) and urinalysis were conducted for 10 rats/sex/group at 6, 12 and 18 months of study and for 20 rats/sex/group at 24 months of study.

Food and water were available to the animals prior to urine collection at 6 months, but were withheld overnight prior to urine collection at 12, 18 and 24 months. Food and water were available prior to blood collection at all test intervals. Blood was obtained by orbital sinus puncture.

Hematologic determinations at all test intervals included hemoglobin, hematocrit, RBC, total and differential WBC, and clotting time. At 12, 18 and 24 months, determinations also included reticulocyte count and partial thromboplastin time.

Biochemical determinations at all test intervals included glucose (non-fasting), BUN, SGPT, SAP, albumin, total protein, bilirubin, cholesterol, sodium, chloride, and potassium, plasma, RBC and brain cholinesterase activities also were determined. At 6 months SGOT was inadvertently performed. At 12, 18 and 24 months, determinations also included calcium, magnesium, uric acid and LDH (total and isoenzymes).

Urinalysis included description of appearance, measurement of volume, pH, and specific gravity; tests for glucose, protein, ketones, bilirubin, and occult blood; and microscopic examination of the sediment.

Interim sacrifices of 10 males and 10 female from each group were conducted at 6, 12, and 18 months after study initiation. After 24 months of study all survivors were sacrificed.

All animals in scheduled and moribund sacrifices and those that died during the study period were necropsied. A thorough necropsy examination was done to include examination of natural orifices, external surfaces and interval organs.

Any gross lesions observed at necropsy were appropriately recorded. During scheduled sacrifices the following organs were weighed: spleen, liver, kidneys, gonads, heart, brain, lungs, adrenals, thyroid and pituitary. From all necropsied animals, masses or gross lesions of indefinite nature were collected and individually identified. In addition the following tissues were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

brain
thoracic spinal cord
pituitary
thyroid/parathyroid
thymus
adrenals
heart
lungs
spleen
liver
kidney
esophagus
stomach
duodenum
ileum
cecum
colon
pancreas
testes with epididymides
prostate
seminal vesicles
salivary gland
mesenteric lymph node
urinary bladder
sciatic nerve with muscle
eyes
bone marrow
sternum
mammary gland
skin

Results:

Findings seen with similar frequency for control and treated rats included soft stool, red material and/or reddened area around the eye, localized hair loss, yellow material on the anogenital region, pale coloration, labored breathing and decubital ulcers of the hind feet.

Masses also were observed quite commonly among control and treated rats. The number of viable rats with masses during week 104 was as follows:

Dosage Level (ppm)	Rats with Masses, Week 104	
	Male	Female
0	13	24
10	16	30
20	8	24
100	11	27

Mortality at week 104 was as follows:

Dosage Level (ppm)	Deaths/Number of Rats Initiated Minus Interim Sac.	
	Male	Female
	37/60	25/60
	22/60	21/60
	33/60	31/60
	21/60	27/60

Through most of the study the mean body weight was slightly lower for male rats at the 100 ppm dosage level than for control rats (Statistically significant, $p < 0.01$, at most intervals of analysis).

However the difference decreased somewhat during the last 6 months of study. The group mean body weights and percent differences from the control group means at 18 and 24 months of study were as follows:

Dosage Level (ppm)	Mean Body Weights, Grams (% difference from control) 18 Months Male	18 Months Female	24 Months Male	24 Months Female
0	790	456	729	495
10	800(+1.3)	477(+4.6)	765(+4.9)	511(+3.2)
20	771(-2.4)	474(+3.9)	751(+3.0)	505(+2.0)
100	702(-11.1)	439(-3.7)	691(-5.2)	452(-8.7)

Food consumption was similar for control and treated rats. No changes considered to be related to the test material were observed during ophthalmoscopic examinations. Hematocrit values were similar for control and treated rats.

No treatment related biochemical effects, excluding cholinesterase depression, were observed during the study. Cholinesterase levels were unaffected at the 10 or 20 ppm exposure levels whem compared to controls. The data for the 100 ppm exposure level is presented below:

Parameter	Study Interval Months	Units of Activity (% depression) Males Control	Males 100 ppm	Females Control	Females 100 ppm
Plasma ChE	6	2.2	1.6(27)**	7.1	6.9 (3)
	12	3.5	2.2(37)**	12.9	9.6(26)*
	18	4.4	3.3(25)*	11.2	9.5(15)
	24	6.0	4.2(30)	11.9	10.7(10)
RBC ChE	6	11.9	10.6(0)	13.9	12.1(13)*
	12	14.2	11.7(18)**	14.6	13.0(11
	18	22.5	17.1(24)*	20.6	18.1(12)*
	24	16.1	13.1(19)**	17.7	14.3(19)**
Brain ChE	6	10.6	10.6(0)	17.1	13.3(22)
	12	10.5	9.3(11)	11.4	9.4(18)
	18	13.9	10.4(25)**	15.4	11.4(26)**
	24	10.5	8.3(21)**	15.1	8.6(43)**

*Significant at $p < 0.05$
**Significant at $p < 0.01$

Urinalysis findings generally were similar for control and treated rats. Analyses of the necropsy data did not reveal a compound-related pattern in mortality or the gross lesions observed. Some statistically significant variations in organ weights were observed. The variations are presented in the following table.

Group	Sex	Organ	Weight	Change	P <	Interpret.
6-Month Interim Sacrifice:						
II	F	brain	relative	decrease	0.05	> b.w.
IV	F	spleen	relative	decrease	0.05	> b.w.
IV	F	kidneys	absolute	increase	0.05	> b.w.
IV	M	lungs	relative	increase	0.05	< b.w.
IV	M	adrenal	relative	decrease	0.05	< b.w.
12-Month Interim Sacrifice						
II	M	lung	absolute	increase	0.01	> b.w.
II	M	pituitary	absolute	increase	0.05	> b.w.
III	M	pituitary	absolute	increase	0.05	?
IV	M	brain	absolute	increase	0.05	no gross
			relative	increase	0.05	lesions
IV	M	adrenal	relative	increase	0.05	< b.w.
IV	M	pituitary	absolute	increase	0.05	no gross
			relative	increase	0.05	lesions
18-Months Interim Sacrifice						
II	M	heart	absolute	decrease	0.05	< b.w.
III	M	heart	relative	decrease	0.05	> b.w.
IV	M	heart	absolute	decrease	0.05	< b.w.
Terminal Sacrifice						
II	F	brain	absolute	increase	0.05	> b.w.
IV	M	lung	absolute	decrease	0.05	< b.w.
IV	M	kidney	absolute	decrease	0.05	< b.w.
IV	F	heart	relative	increase	0.05	< b.w.
IV	F	brain	absolute	increase	0.01	no gross
			relative	increase	0.05	lesions

* > b.w. = due to increase in body weight
< b.w. = due to decrease in body weight
? = significance unknown.

Most of these organ weight variations are due to reductions in body weight as indicated under the interpretation column. Reduction in body weights of Group IV males is apparent in all the interim and the terminal sacrifices whereas the Group IV females show a reduction in body weight only at the terminal sacrifice. An increase in the absolute and relative weight of brain and pituitary was evident in Group IV males at the 12-month interim sacrifice. No gross lesion was evident to explain this increase.

However, a similar change in brain weight of Group III males, was not observed at the 18-month and terminal sacrifices, hence its significance should be taken as unknown particularly in the absence of histopathologic findings. On the contrary, the Group IV females showed an increased absolute and relative brain weight at terminal sacrifice but not at terminal sacrifice but not at interim sacrifices. These animals also did not reveal any gross lesion in brain to explain this change. In the absence of histopathologic findings, it significance is unknown.

Neoplasms and non-neoplastic histologic changes, by type, incidence, and/or degree of severity, in rats which died, killed moribund, and those killed following 24-months of study were considered to represent spontaneous lesions and to be unrelated to the administration of or exposure to the test material.

Conclusion:

Oncogenic potential is negative. Systemic NOEL is 20 ppm. Cholinesterase NOEL is 20 ppm. The LEL for Cholinesterase inhibition and systemic effects is 100 ppm.

Classification: Core-Guidelines

2) 2-Year Dietary Toxicity and Carcinogenicity Study in Mice (IRDC Report#164-116; Jan. 4, 1980)

Test Material: Carbofuran tech., 95.6%, Furadan Insecticide MrS1314, 5 lbs., 3/16/77; brown fibrous powder with very dark brown or black chunks.

Four hundred male (20-30 gms) and 400 female (18-29 gm) Charles River CD-1 mice were initiated on test. Five additional mice/sex/group were initiated in the study as possible replacement animals. A male (28677: Group II) and a female (28972: Group III) that died during week 1 and 3, respectively, were replaced. All other replacement mice were sacrificed and discarded at the end of the 4-week replacement period.

The mice were housed inidividually in suspended wire-mesh cages and maintained in a temperature-, humidity-, and light- (12-hr. light/12 hr. dark) controlled room. Food and water were available ad libitum. The randomly selected and assigned mice were distributed as follows:

Group	Treatment (ppm)	No. Initiated/Group Males	Females
I	0 (control)	100	100
II	20	100	100
III	125	100	100
IV	500	100	100

Each animal was observed three times daily (Monday-Friday) or twice daily (weekends and holidays) for signs of overt toxicity, moribundity and mortality. Detailed examinations were conducted weekly. Mortality was recorded on the day the animal died. Body weights on all mice were measured and recorded weekly. Fresh food and/or the respective test diet was presented weekly. Food and test diet consumptions were measured and recorded at the time of feed change.

During treatment months 6, 12, 18 and 24, the following samples were obtained for analyses. Animals randomly selected for collection of samples and necropsy were fasted sons water overnight for collection of urine samples prior to sacrifice by exsanguination and immediate necropsy. Five non-fasted animals/sex/group were sacrificed each day of the scheduled sacrifice period to assume proper measurement of brain cholinesterase and adequate blood for hematology and biochemistry.

Hematologic studies included: hematocrit, hemoglobin, total RBC count, total and differential WBC count and clotting times.

Biochemical analysis of non-fasted serum samples included: BUN, SAP, SGPT glucose (6 and 12 months) and albumin (12, 18 and 24 months). Glucose determinations were discontinued after the 12-month sampling as per protocol.

Urine samples obtained from fasted mice were used for urinalysis which included: description of appearance, pH, specific gravity, protein, glucose, occult blood, bilirubin, ketones, and microscopic examination of spleen deposit.

Cholinesterase activity was determined on brain tissue at the sacrifice of the animals selected for the clinical laboratory determinations. The animals were non-fasted prior to sacrifice by exsanguination and were sacrificed in the mornings of consecutive days.

Ten male and 10 female mice from each group were sacrificed and necropsied after 6, 12, adn 18 months of study. At necropsy, an examination was made of the external body surfaces adn orifices. Each mouse was then opened and contents of the body cavities were examined for gross abnormalities, selected organs were weighed (heart, lungs, spleen, liver, kidneys, gonads, pituitary, adrenals, brain, thyroid/parathyroids) and tissues collected for fixation in buffered neutral 10% formalin.

Mice that died or were sacrificed in extremis during the course of study were also necropsied and tissues collected as above.

The specified tissues that were examined microscopically from all mice of all groups included the adrenal glands, bone marrow, brain, cecum, cervix, colon, duodenum, middle ear, epididymes, esophagus, eyes, gall bladder, lacrimal/Harderian gland, heart, ileum, jejunum, kidneys, liver, lungs, cervical and mesenteric lymoh nodes, mammary gland, nasal cavity, ovaries, pancreas, parathyroid, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, cervical and thoracic spinal cord, spleen, stomach, testes, thyroid, thymus, trachea, urinary bladder, uterine horns and body and all tissues with gross lesions.

The tissue microslides were prepared by American Histolabs, Inc. of Rockville, Maryland, and the microscopic slides were submitted to Research Pathology Services, Inc. for the microscopic evaluation.

Results:

No changes in appearance or behavior that could be definitely attributed to the test material were noted for any mice throughout the conduct of this study. Mortality of the animals was not indicative of a dosage relationship. The greatest mortality was noted for the low-dosage-level males and the control females.

Survival at 104 weeks was:

Group (ppm)	No. of Survivors/No. Initiated less interim sacrifice	
	Males	Females
0 (control)	33/70	24/70
20	31/70	40/70
125	46/70	40/70
500	38/70	36/70

The sex group mean body weights of the treated groups were similar to the sex-group mean body weight of the controls by the end of the study. Through week 65 body weight analyses, however, a dosage-related decrease was evident for the male mice but less evident for the females.

At the high-doage level the differences from the controls were statistically significant ($p < 0.01$) for the males at 13, 26, 39, 52 and 65 weeks and were statistically significant ($p < 0.01$ or $p < 0.05$) for the females at 26, 39, 52 adn 78 weeks.

At week 104 no statistical differences were noted for treated and control; however, high-dosage-level group means were slightly lower than controls. Group mean body weights at 104 weeks as follows:

Treatment Level (ppm)	Mean Body Weight, Gm.	
	Male	Female
0 (control)	38.3	34.2
20	38.3	35.8
125	39.1	33.9
500	37.5	32.6

Food consumption was very slightly lower for the treated animals when compared by sex, to the controls for all time intervals (except at week 104) statistically. The only statistically significant differences were for the week 1-13 period at the high-dosage level.

No specific differences that were related to compound were noted in the hematologic parameters measured at the prescribed time intervals. With the exception of the brain cholinesterase values, no other values for the parameters measured were indicative of a dose-related effect.

Brain cholinesterase levels were significantly decreased for treated males and females at the 125 and 500 ppm dosage levels when compared with the controls at all time intervals. No effect on brain cholinesterase levels at the 20 ppm dosage level was observed at any of the intervals of analysis.

No specific trend nor evidence of a compound-related effect in urinalyses was noted for the parameters measured.

The gross pathology lesions described at necropsy from all groups of mice that were either sacrificed or died during the course of study were considered of spontaneous nature, not uncommon to mice of this strain, and unrelated to compound administration.

Statistically significant mean weight variations occurred in few organs among mice at each dose level during the three interim and terminal sacrifices. In the absence of any histopathologic effects, no biological significance can be attached to these organ weight variations.

No treatment-related microscopic chanegs were observed in any of the carbofuran-treated mice. Also, treatment with the compound had no tumorigenic influence, as there were no variations in tumor incidence that could be correlated with compound administration.

The various non-neoplastic changes observed in the mice of the various treatment groups were of the type that commonly occur as spontaneous changes in aged Charles River CD-1 mice and there was no alteration in the incidence of these findings that could be correlated with treatment. The various benign and malignant neoplasms also occurred at similar incidences in the control and treated groups.

Conclusion:

Oncogenic potential is negative. Systemic NOEL is 125 ppm. Cholinesterase NOEL is 20 ppm.

Classification: Core-Guideline

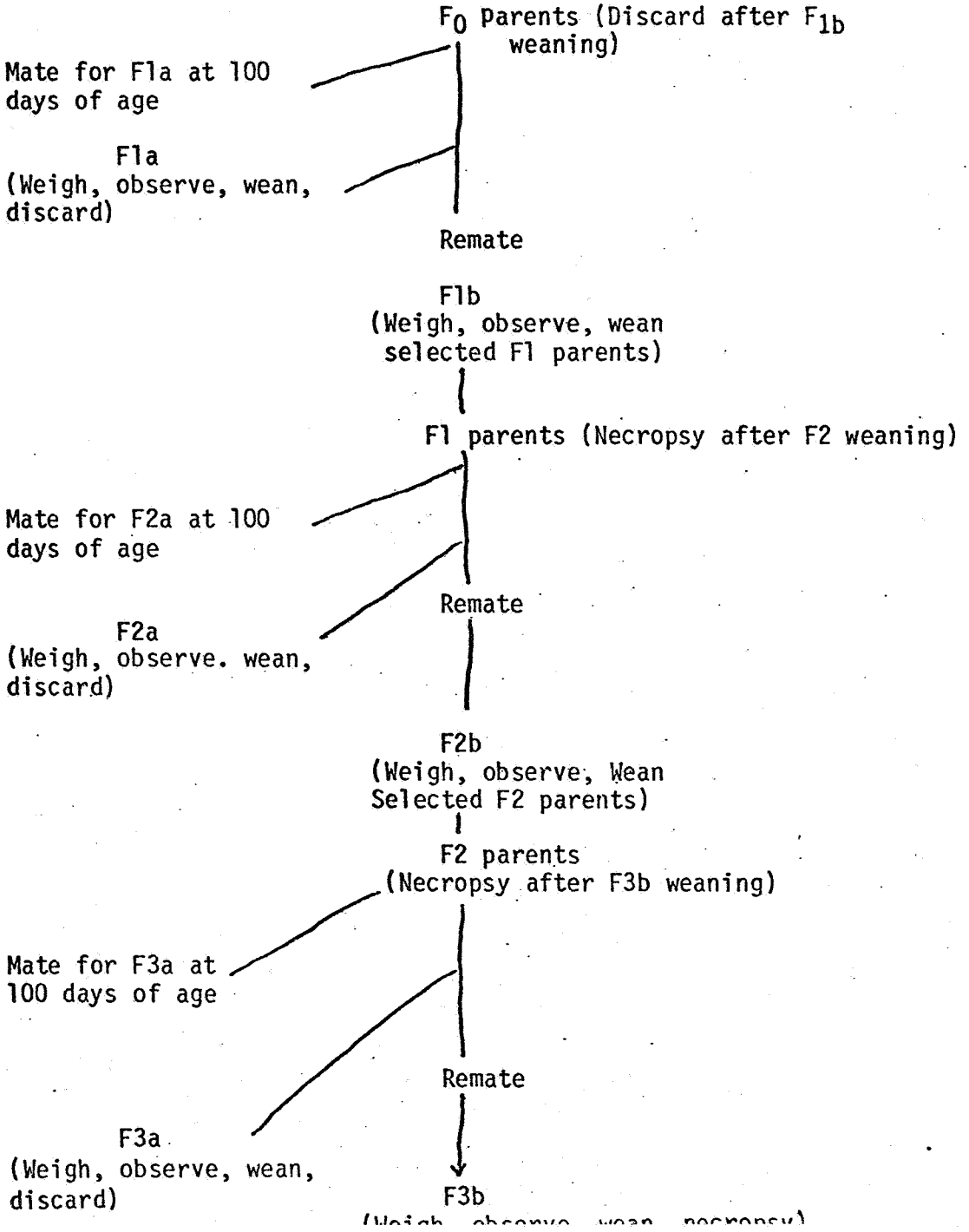
3) Three-Generation Reproduction Study in Rats (IRDC Report#167-114; November 9, 1979)

Test Material: Carbofuran tech., 95.6% Furadan Insecticide; MrS1314, 5 lbs, 3/16/77; brown fibrous powder with very dark brown or black chunks.

Carbofuran technical in an acetone vehicle was administered in basal diet at dosage levels of 20 and 100 ppm to Charles River CD rats. A control received only acetone in the diet.

The first (F_0) and second (F_1) generations each had 10 male and 20 female parental rats in each of the two treatment groups and one control group. The third (F_2) generation parents numbered 12 males and 24 females per group. Each generation was mated twice to produce two litters as shown in the following scheme:

Three-Generation Reproduction Study Rats Bleeding Schematic



The parental rats and pups were observed daily for signs of overt toxicity, changes in general behavior and appearance and mortality.

Detailed observations, individual body weights and food consumption were recorded on a weekly basis for the parental rats. In addition, the F1 and F2 parental females were weighed on days 0, 7, 14 and 21 of generation and on days 0, 4, 14, and 21 of lactation.

Specific observations for the reproductive aspects of this study included male and female fertility, length of the generation periods, litter size and growth, viability and survival of the pups through weaning. The pups were weighed as litters on lactation days 0, 4, and 14 and individually, by sex, on lactation day 21. During the F1 and F2 generations, the numbers of male and female pups at lactation days 0, 4, 14 and 21 were recorded. Litter size was reduced to 10 pups of equal sex ratio, if possible, on day 4 of lactation.

Results:

No changes considered to be related to treatment of Carbofuran technical were seen in the general behavior appearance or survival of the treated parental rats when compared to the control rats.

No differences were seem between the litters in the control and treated groups with regard to general behavior or survival which was considered treatment related.

Dehydration was noted in pups from four litters of the F3a and three litters of the F3b at the 100 ppm treatment level. The appearance of the pups in the 20 ppm treatment group was similar to the control.

Mean body weights of the parental rats receiving Carbofuran technical at 20 ppm were not affected by treatment. The mean body weight of the 20 ppm F_1 females at study week 28 was statistically significantly higher than the control females.

This difference was not considered a treatment effect due to lack of any similar results during the F_0 and F_2 generations.

At 100 ppm mean body weights of the parental rats were consistently lower than the control weights throughout treatment. The mean body weights of the males at study week 28 (F_0), study weeks 37 and 57 (F_1) and study weeks 67 and 87 (F_2) were statistically significantly lower than the control group. In addition, significantly lower body weights were seen for the females at study week 8 (F_0) and study weeks 58 and 67 (F_2). Female body weights during the F_{2a}, F_{2b}, F_{3a} and F_{3a} gestation adn lactation periods at the 100 ppm treatment level were lower than the control group.

The generation and lactation body weights of the 20 ppm F_1 and F_2 females were comparable to the control females.

Food consumption values of the 100 ppm males of all three generations and the 100 ppm F_0 females were generally lower than their respective controls. No treatment-related food consumption differences were seen between the 20 ppm F_0, F_1, and F_2 males and females, the 100 ppm F_1 and F_2 females and the control rats.

No treatment-related differences were seen between the control and treated parental rats with respect to male and female fertility and length of the gestation periods.

The growth, viability and survival of the pups in the 20 ppm treatment group were similar to the control with one exception. The numbers of liveborn pups in the F_{3a} litters were statistically significantly lower than the control group. This difference was not considered biologically meaningful and was attributed to random occurrence. The mean pup body weights of the litters in the 20 ppm treatment group were generally similar to the control group.

In the 100 ppm treatment group, the survival of the F_{1a}, F_{2a} and F_{3a} pups at lactation day 4 was slightly lower than the control. In the F_{3a} litters, one litter contributed 14 of the 26 deaths.

At this same treatment level, the mean pup body weights at lactation days 0, 4, 14 and 21 were consistently lower than the control group. At lactation day 21, the combined male and female pup body weights of each of the F_{1a}, F_{1b}, F_{2b}, F_{3a}, and F_{3b} litters were statistically significantly lower than the control litters.

No pathological lesions or abnormalities which were considered compound-related were seen at necropsy in any F_0, F_1, F_2 parental rats and pups from the F_{2b} and F_{3b} litters sacrificed at termination or in pups or parental rats necropsied during the course of study.

The lesions decribed among these rats were considered of spontaneous occurrence and not compound related.

Statistically significant mean weight variations occurred in various organs among the F_2 Parental and F_{3b} Weanling rats sacrificed at termination of study at the 20 and 100 ppm dosage levels.

Organ	Dosage Level (ppm)	Sex	Weight	Change	p <
F_2 Parental Rats, Terminal Sacrifice:					
spleen	100	M	absolute	decrease	0.05
	100	F	absolute, relative	decrease	0.01
liver	20	M	relative	decrease	0.05
	100	M	absolute, relative	decrease	0.05
ovaries	100	F	absolute, relative	increase	0.01
adrenals	20	M	absolute, relative	decrease	0.01
	100	M	absolute	decrease	0.01
thyroid	20	M	absolute, relative	decrease	0.01
F_{3b} Weanling Rats, Terminal Sacrifice:					
spleen	100	M	absolute	decrease	0.01
	100	F	absolute, relative	decrease	0.01, 0.05
liver	100	M	absolute, relative	decrease	0.01
	100	F	absolute, relative	decrease	0.01, 0.05
kidneys	100	M	absolute	decrease	0.01
	100	F	absolute, relative	decr. & incr.	0.01
ovaries	20	F	absolute, relative	increase	0.01
	100	F	relative	increase	0.05
testes	100	M	absolute	decrease	0.05
heart	100	M	absolute	decrease	0.01
	100	F	absolute	decrease	0.01
adrenals	20	F	relative	increase	0.05
	100	M	relative	increase	0.01
	100	F	absolute	decrease	0.05
thyroid	20	F	absolute, relative	increase	0.01

Random variation in organ weights frequently occur in toxicity studies.

Conclusion: The NOEL for reproductive parameters is 20 ppm.

Classification: Core-Minimum DATA

TS-769:th:LCHITLIK:8-14-80
3/14/81