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DATA EVALUATION REPORT

STUDY TYPE: Multigeneration Reproduction - Rat (Guideline 83-4)

Tox Chem No: 717C
MRID No: 416956-01

TEST MATERIAL: Dithiopyr, technical grade (stated purity of 93.7%) described as a Dayton Batch No. 1.

SYNONYMS: MON-15100; MON-7200; S,S-Dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine dicarbothioate.

SPONSOR: Monsanto Agricultural Company

TESTING FACILITY:
The Institute of Environmental Toxicology, Kodaira, Tokyo 187, Japan

LABORATORY PROJECT NO.:
IET 87-0053/ET-88-3 - IET87-0004/ET-87-154

REPORT TITLE: Dithiopyr (MON-7200) Reproduction Studies in Rats Part I. Two-Generation Reproduction Study in Rats with MON-7200

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REPORT ISSUED: November 28, 1989

CONCLUSIONS:

Parental Toxicity:

NOEL = 25 ppm (1.7 mg/kg/day in males; 1.91 mg/kg/day in females).

LEL = 250 ppm (16.4 mg/kg/day in males; 18.6 mg/kg/day in females) based on decreased body weight gain (F1 males), increased relative liver weight (F0 males and F1 females), and increased absolute kidney weight (F0 females).
Histopathological evidence of liver, kidney, thyroid, and adrenal toxicity in parental animals was observed at 2500 ppm, the high dose group (both sexes, both generations).

Reproductive Toxicity:

NOEL = equal to or greater than 2500 ppm, the highest dose tested in F0 and F1 parental animals.

No compound-related adverse effects were observed on reproductive parameters such as mating, fertility, gestation indices, and length of gestation.

Offspring Toxicity:

Body weights of F1 and F2 pups (both sexes) in the high dose group were decreased as compared to controls. Gross examination revealed liver enlargement in F1 and F2 weanlings (both sexes) in the high dose group. Histopathological examination of the liver revealed diffuse hepatocellular swelling at 250 ppm, the mid dose group (both sexes, F1 and F2 pups) and at 2500 ppm, the high dose group (both sexes, both generations). Liver toxicity observed was a significantly increased incidence of 'white spots' located on the outer margins of livers of F1 and F2 mid dose male pups culled on day 4 of lactation as compared to controls. The incidence was also found in pups and weanlings (both sexes, both generations) of the high dose group. There were no indications of treatment related effects on mean number of pups/litter, sex ratio or pup viability in both generations.

Dose levels in the feed tested: 0, 25, 250, or 2500 ppm
Test species [strain]: rat [Charles River Crj:CD (SD)]

CORE CLASSIFICATION: Core minimum. This study satisfies the guideline requirements for a two-generation reproduction study (83-4) in rats.

I. MATERIALS

Test Compound
Purity: 93.7%
Description: a light yellow solid
Odor: very faint sulfur odor
Lot No.: Dayton Batch No.1
Melting point: 48-51° C
Vapor pressure: 4 x 10^{-6} torr at 25° C
Solubility: 0.7 ppm in water at 25° C. Soluble in methanol, acetone, and chloroform

Vehicle
None used; the test material was administered in the diet

Test Animal(s)
Species: Rat
Strain: Charles River Crj:CD (SD)
Source: Charles River Japan Inc.
Age: 5 weeks at the start of the study
Acclimation Period: 8 days

Environmental Conditions
Temperature: 24±1° C
Humidity: 55±10%
Light:dark cycle: 14:10

(lights on at 5 a.m. and off at 7 p.m.)

Food and Water

Food (Oriental Yeast Co., Ltd.) and water were routinely analyzed for suspected contaminants. The study reported that no contaminants were detected in such levels that could adversely affect the results of the study.

Diet preparation

The test substance was dissolved in acetone and then mixed with a part of the basal feed to make a pre-mixture. The pre-mixture and the basal feed was used to make a test diet of the prescribed concentration. The same amount of acetone was also added to the control diet. After mixing, the prepared diets were retained for about 30 minutes in a draft chamber to evaporate acetone. Test diets were analyzed for homogeneity prior to the initiation of the study. Stability of the test substance in the test diet was examined after storing the test diets for six weeks at room temperature. The concentration of the test substance in the test diets was analyzed at 8 different intervals during the study.

II. PROCEDURES AND STUDY DESIGN

Study Design

This study was designed to assess the potential effects of MN-7200 through continuous dietary administration on reproductive performance in rats for two successive generations.

Group Arrangement

After quarantine and acclimation, 5-week-old animals were assigned to 4 experimental groups following a computerized randomization procedure based on body weight as follows:

Test groups		Dose	Animals per group **	
No.	Designation	(ppm) *	Males	Females
1	Control	0	24	24
2	Low (LDT)	25	24	24
3	Mid (MDT)	250	24	24
4	High (HDT)	2500	24	24

* Diets were administered from the beginning of the study until the animals were sacrificed.

** The same number of animals were picked from the F1 litters as parents for the F2 generation.

The experimental outline for this study is shown in Figure 1 (attached). The animals were fed for 11 weeks (premating growth period) and the mating of animals was initiated. At the beginning of treatment week 12, females with proestrus vaginal smears were cohabited overnight with males of the same group on a 1:1 basis. On the following morning, the females were examined for the presence of vaginal plugs and/or sperm in order to ascertain whether copulations had occurred. If evidence of copulation was found, the day was designated as day 0 of gestation. Copulated females were placed individually into breeding boxes. Females not showing any evidence of copulation were returned to their cages and re-examined daily for the estrous cycle. Females with proestrus vaginal smears were re-mated with the same males. The procedures were repeated within the 3-week mating period.

The day on which delivery was completed was designated as day 0 of lactation. On day 4 of lactation, pups were selected by sex at random so that the number of pups in a litter would be 8 (if possible, 4 males and 4 females), and the remaining pups were killed and discarded after gross autopsy. If the number of pups in a litter was 8 or less, all the pups were nursed. Pups were weaned on day 21 of lactation.

In each group, 24 males and 24 females were selected from F1 weanlings at 21-26 days of age to become F1 parental animals. Since it was difficult to equalize the mean body weights among groups, one or 2 pups were selected from each litter through computerized randomization procedure for potential F1 parental animals. The remaining F1 weanlings were killed, autopsied and their livers were fixed and preserved in 10% neutral-buffered formalin.

F1 parental animals were fed for 11 weeks and bred to obtain F2 animals in the same manner as described for F0 parental animals. Sibling matings were avoided. After weaning of F2 pups all animals, including F1 parental animals, were killed and autopsied. The livers from all the F2 offspring which died or were killed on the study were fixed and preserved in 10% neutral-buffered formalin.

In the F0 and F1 generations, a pair of animals that failed to mate during the 3-week mating period and females that did not show any evidence of parturition by 25 days after copulation were killed and macroscopically examined at the time of autopsy of the other parental animals.

Observations

Observations and the schedule for those observations are summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	All	Twice a day during premating and growth periods.
Body weight	All	At beginning of study and weekly through growth and mating periods.
	Maternal animals	Days 0, 7, 14, and 20 of gestation; days 0, 7, 14, and 21 postpartum; and on the day of autopsy.
	Paternal animals	Once per two weeks during breeding period, and on the day of autopsy.
Food consumption	All	Weekly during premating period.
	Maternal animals	Days 0, 7, 14, and 20 of gestation; days 0, 7, 14, and 21 postpartum;
	Paternal animals	Once per two weeks during breeding period.

(1) Parental animals

1-a Clinical signs and mortality

Animals were examined for their general condition twice a day on Monday through Friday except holiday and once a day on Saturday, Sunday, and holiday. At time of weighing, they were subjected to complete external examination and any abnormalities were recorded. Animals in moribund condition or found dead were autopsied immediately after discovery and any pathological findings were recorded.

1-b Body weights

For males, the determination was made at initiation of treatment, weekly during the premating growth period, once per two weeks during the breeding period, and on the day of autopsy. For females, the determination was made at initiation of treatment, weekly during the premating growth period, on days 0, 7, 14, and 20 of gestation and on days 0, 7, 14, and 21 postpartum and on the day of autopsy.

1-c Food consumption

During the premating growth period, total food consumption was determined weekly for both sexes. During the breeding period (except the mating period during which no determination was made) total food consumption was determined once per two weeks for males. For females, total food consumption was determined at intervals of days 0-7, days 7-14, and days 14-20 of gestation and of days 0-7, days 7-14, and days 14-21 of lactation. Food consumption of females during the lactation period was expressed as the total amounts of food consumed by maternal animals and their offspring.

1-d Chemical intake

Chemical intakes of both sexes of the treated groups were calculated during the premating growth period on a weekly basis from the following formula:

$$\text{Chemical intake (mg/kg/day)} = \frac{\text{mean food consumption (kg/day)} \times \text{dietary level (mg/kg)}}{\text{mean body weight (kg)}}$$

The values were expressed as mg MON 7200/kg body weight/day.

1-e Reproductive performance

1) Estrous cycle

Vaginal smears were taken from each female, stained with Giemsa solution, and examined microscopically. Regularity in microscopic changes of vaginal smears associated with various stages of the estrous cycle was checked for one week or more.

2) Mating index

Copulation was confirmed by the presence of vaginal plugs and/or sperm in vaginal smears. Mating indices were calculated for both sexes by the following formulae:

$$\text{Male mating index (\%)} = \frac{\text{number of copulations}}{\text{number of males used}} \times 100$$

$$\text{Female mating index (\%)} = \frac{\text{number of copulations}}{\text{number of females used}} \times 100$$

3) Fertility index

Pregnancy was confirmed by the occurrence of parturition or by the presence of implantation sites in the uterine horns at necropsy. The fertility index was calculated from the following formula:

$$\text{Fertility index (\%)} = \frac{\text{number of pregnancies}}{\text{number of copulations}} \times 100$$

4) Gestation index

Parturition resulting in birth of one or more live pups was considered to be normal. The gestation index was calculated from the following formula:

$$\text{Gestation index (\%)} = \frac{\text{number of normal parturitions}}{\text{number of pregnancies}} \times 100$$

5) Duration of gestation

Duration of gestation was represented as the number of days from detection of copulation to completion of parturition.

1-f Pathological examination

1) Gross pathological examination

All FO and F1 parental animals were killed and autopsied after the weaning of their offspring. Any gross pathological findings were recorded. The following organs and tissues were fixed and preserved in 10% neutral-buffered formalin.

Brain
Spinal cord (cervical, thoracic, and lumbar regions)
Peripheral nerve (sciatic nerve, unilateral)
Pituitary
Thymus
Thyroids with parathyroids
Adrenals
Spleen
Bone with marrow (sternum, femur)
Tibia-femoral joint (unilateral)
Lymph nodes (cervical, mesenteric)
Heart Aorta

Salivary glands	Tongue
Esophagus	
Stomach (forestomach and glandular stomach)	
Liver Pancreas	
Duodenum	Jejunum
Ileum	Cecum
Colon	Rectum
Head (including nasal cavity, paranasal sinuses, buccal mucosa and middle ears)	
Pharynx	Larynx
Trachea	Lung
Kidneys	Urinary bladder
Testes	Epididymides
Prostate	
Seminal vesicles and coagulating glands	
Ovaries	
Uterus	
Eyes and Harderian glands	
Skeletal muscle (M. triceps surae, unilateral)	
Skin (lumbodorsal region)	
Mammary gland (abdominal region; females only)	
All gross lesions	

2) Organ weights

Ten male and 10 female parental animals in each group from the FO and Fl generations were randomly selected and the following organs from these animals were weighed before fixation: brain, heart, liver, kidneys, spleen, adrenals, epididymides, ovaries, and testes.

3) Histopathological examination

Histopathological examination was conducted on the organs and tissues of parental animals which had shown some abnormalities at necropsy. Males and females which had failed to produce offspring were also examined histopathologically for abnormalities of the reproductive organs, i.e., ovaries, uterus, and vagina for females and testes, epididymides, seminal vesicles, and prostate for males. In the high dose and control groups, all FO and Fl parental animals were examined histopathologically for abnormalities of the reproductive organs including the pituitary. In addition to these organs, the liver, kidneys, thyroids and adrenals were examined histopathologically on all parental animals of all dose groups because test substance-related abnormalities were observed in these four additional organs in the 2500 ppm group.

(2) Offspring

2-a Clinical signs and mortality

All pups were observed daily during the lactation period for their clinical signs. At time of weighing, they were subjected to complete external examination and any abnormalities were recorded. Pups found dead were autopsied immediately after discovery and any findings were recorded.

2-b Mean number of pups delivered

For each litter delivered normally, live and dead pups were counted on day 0 of lactation. The number of pups delivered was expressed as the sum of live and dead pups. Group mean values were calculated from the following formula:

$$\text{Mean number of pups delivered} = \frac{\text{total number of pups delivered}}{\text{number of normal parturitions}}$$

2-c Sex ratio

All pups were sexed on day 0 of lactation and then sex ratio was calculated for each group from the following formula:

$$\text{Sex ratio} = \frac{\text{total number of male pups}}{\text{total number of pups delivered}}$$

2-d Viability index

Viability indices on days 0, 4, and 21 of lactation were calculated for each litter from the following formulae and then group mean values were calculated using the individual litter values.

Viability index on day 0 of lactation (%)

$$= \frac{\text{number of pups alive on day 0 of lactation}}{\text{number of pups delivered}} \times 100$$

Viability index on day 4 of lactation (%)

$$= \frac{\text{number of pups alive on day 4 of lactation}}{\text{number of pups alive on day 0 of lactation}} \times 100$$

Viability index on day 21 of lactation (%)

$$= \frac{\text{number of pups alive on day 21 of lactation}}{\text{number of pups selected on day 4 of lactation}} \times 100$$

2-e Body weights

In each litter, all live pups were weighed by sex on days 0, 4, 7, 14, and 21 of lactation.

2-f Pathological examination

1) Gross pathological examination

F1 and F2 pups not selected on day 4 of lactation were subjected to autopsy. F1 weanlings not selected for F1 parental animals and all F2 weanlings were killed and autopsied at 21-27 days of age. All pathological findings were recorded. In addition, the livers of above-mentioned animals except for F1 pups were fixed and preserved in 10% neutral-buffered formalin because unusually high incidence of white spots were observed in F1 cull livers.

2) Histopathological examination

Histopathological examination was conducted on all pup and weanling livers displaying white spots (except F1 pups). In addition, the livers from randomly selected F2 culls and F1 and F2 weanlings (5/sex/group) were examined histopathologically. The livers from F2 pups dying spontaneously were also examined.

Statistical Evaluations:

The following statistical tests were used to estimate significance of the differences between the control group and each of the treated groups.

Equality of variances was evaluated by Bartlett's test. When group variances were homogeneous, a parametric analysis of variance in one-way classifications was used to determine if any significant differences existed among groups. If the analysis of variance was significant, Dunnett's test or Scheffe's multiple comparison test was performed to detect any statistically significant differences between the treatment groups and their corresponding controls. When Bartlett's test indicated that the variances were not homogeneous, the Kruskal-Wallis nonparametric analysis of variance was used for detecting any statistical differences among groups and if significant, Dunnett's type mean rank test or Scheffe's type mean rank test was performed to detect the statistical differences between the treatment groups and their corresponding controls. Fisher's exact probability test was used for analysis of parental mating, fertility, and gestation indices, the sex ratio of offspring, and for the incidence of pathological findings. Mann-Whitney's U-test was used for analysis of data on the duration of gestation and on the

viability indices of pups. Significance of the differences between the control groups and the treated groups was estimated at 5 and 1% levels of probability.

Compliance:

A signed Statement of Confidentiality Claim was provided.
A signed Statement of compliance with EPA GLP's was provided.
A signed Quality Assurance Statement was provided.

III. RESULTS

A. Analysis of test diets

Stability analysis showed that diets stored for 6 weeks at room temperature were within 2% of the target concentration. The diet mixtures were shown to be homogeneous (dietary levels ranged from 90 to 97% of target concentrations) for all dose levels sampled from the top, middle and bottom of a mixture. The overall means of concentration of test substance were within 6% of the target concentration for all dose levels.

B. (1) Parental animals

1-a Mortality and clinical signs: No treatment related clinical signs were observed in animals of either sex in any treated groups throughout the treatment period. Although some animals were found dead or killed in extremis due to malocclusion, elongated incisors, body weight loss, hypoactivity, respiratory disorder in both generations, these deaths were not considered to be treatment related. These results are summarized from the report as follows:

		Dose group			
Generation	Findings	Control	Low	Mid	High
F0 males	Found dead	1	1	0	0
	Killed in extremis	0	1	1	1
F0 females	Found dead	0	0	0	1
	Killed in extremis	0	0	0	1
F1 males	Found dead	0	0	0	0
	Killed in extremis	0	1	0	1
F1 females	Found dead	0	0	0	0
	Killed in extremis	0	0	1	0

1-b Body weights and body weight gain (Tables 1 and 2 attached):

Males

In the 25 ppm group in the F0 and F1 generations, the body weights and body weight gain of males were similar to

controls. In the 250 ppm group in the F1 generation, body weights were significantly lower (6-7%) at weeks 4 through 13 as compared to controls and body weight gain was significantly lower at week 1 (11%) and weeks 4 through 11 (6-7%) as compared to controls.
In the 2500 ppm group in the F0 generation, the body weights were significantly lower (7-11%) as compared to controls at weeks 11 through 19. In the 2500 ppm group in the F1 generation, the body weights were significantly lower (8-13%) as compared to controls throughout the treatment period. In the F0 generation, the body weight gain was significantly higher (11%) as compared to controls at week 1, but significantly lower (7-11%) than controls at weeks 11 and 19. In the F1 generation, the body weight gains were significantly lower (6-11%) than controls throughout the entire treatment period except for the value at week 2.

Females
In the 25 ppm group in the F0 and F1 generations, the body weight and body weight gain of females were similar to controls. In the 250 ppm group in the F1 generation, the body weights were significantly lower (7-8%) as compared to controls at weeks 4 and 5.
In the 2500 ppm group in the F0 generation, the body weight was significantly higher (4%) as compared to controls at week 1 and the body weight gain was significantly higher (17%) at week 1. In the F1 generation, the body weights were significantly lower (8-18%) as compared to controls throughout the entire treatment period except for gestation day 20 and lactation day 21. The body weight gain in the F1 generation was significantly lower (5-14%) as compared to controls at weeks 1, 2, 6, and 7.

1-c Food consumption (Table 3 attached):

Males

In the F0 generation, food consumption values were significantly higher as compared to controls at week 4 (4%) in the 25 ppm group. In the F1 generation, no significant differences were found in the 25 ppm group. In the 250 ppm group in the F1 generation, the values were significantly lower (5-11%) as compared to controls at weeks 3, 4, and 6. In the 2500 ppm group in the F0 generation, the value was significantly higher (8%) as compared to control at week 11. However, values in the 2500 group in the F1 generation were comparable to those in the control group except for a significantly lower value (8%) at week 1.

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Females

In the 25 ppm group in the F0 generation, food consumption was significantly higher (12%) as compared to controls at week 11. In the 250 ppm group in the F1 generation, food consumption was significantly lower (5-8%) as compared to controls at weeks 1, 2, and 4. In the 2500 ppm group in the F1 generation, food consumption was significantly lower as compared to controls at weeks 1, 2, 4-7, and 11 (5-17% lower) and lactation days 14 and 21 (5% lower).

1-d Chemical intake:

The average chemical intake calculated from the data on food consumption, dose level, and body weight during the pre-mating growth period were as follows:

		Average chemical intake (mg/kg/day)	
Dose (ppm)		Male	Female
25	F0	1.70	1.91
	F1	2.00	2.26
250	F0	16.4	18.6
	F1	19.9	22.5
2500	F0	170	187
	F1	218	230

1-e Reproductive performance (Table 4 attached):

No compound-related adverse effects were observed on reproductive parameters such as mating, fertility, gestation indices, and length of gestation in F0 and F1 parental animals.

1-f Pathological examination:

Gross findings
In the 2500 ppm group, enlargement and discoloration of the liver were observed in virtually all animals of both sexes in both generations. These alterations were not noted in the lower dose groups. No gross changes were found in other organs in any of the treated groups.

Organ weights (Table 5 attached)
In males, no test substance-related changes were observed in the 25 ppm group. In the 250 ppm group, relative liver weight was significantly increased (12%) as compared to

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controls in the F0 generation. In the 2500 ppm group, absolute and relative liver weights were significantly increased in both generations. Absolute liver weights were increased 60 and 64% of controls and relative liver weights were increased 80 and 90% of controls in the F0 and F1 generations, respectively. Absolute and relative kidney weights were increased 14 and 28% of controls in F0 generation, respectively and relative kidney weight was significantly increased (28% of controls) in F1 generation. Although significant decreases in the absolute weights of the spleen (19% of controls) and adrenals (10% of controls) were observed in the F0 and F1 generations, respectively, there were no statistical differences in the corresponding relative values. Relative weights were increased in the brain (14-15% of controls), heart (16%), and testes (15-20%) in both generations. However, these changes were considered to be attributed to the lower body weights.

In females, no test substance-related changes were observed in the 25 ppm group. In the 250 ppm group, absolute liver weight showed a tendency to increase (13-19% of controls) in both generations and relative liver weight was significantly increased (12% of controls) in the F1 generation. Absolute kidney weight was significantly increased (12%) as compared to controls in the F0 generation, however, the difference in their relative weights was not statistically significant. In the 2500 ppm group in both generations, absolute and relative liver weights increased 59-69% and 66-73% of controls, respectively. Absolute and relative kidney weights increased 15 and 12%, respectively of controls in the F0 generation, whereas no statistical differences were found in either weight of the kidneys in the F1 generation. Although significant decrease (16% of controls) in the absolute spleen weight was observed in the F1 generation, no significant difference was found in the relative value.

Histopathological findings (Table 6 attached)

In the 25 ppm group of both sexes in both generations, there were no significant findings in the liver, kidneys, thyroids, and adrenals. In the 250 ppm group, a significantly increased incidence (33% of animals) of cortical cell hypertrophy in the adrenals was observed in F0 parental females. In the 2500 ppm group, a significantly increased incidence (96-100% of animals) of diffuse hepatocellular swelling in the liver was observed in animals of both sexes in both generations. F0 males showed a significantly increased incidence (25% of animals) of focal hepatocellular necrosis in the liver. In addition, there was an increased incidence of bile stasis in the interlobular bile ducts in animals of both sexes in both

generations (25% of the males and 13% of the females in F0 generation; 83 % of the males and 88% of the females in F1 generation). In the kidneys, the incidence of focal tubular atrophy was significantly increased in the F0 generation (96% of the males and 42% of the females) and in the F1 generation (79% of the females). Although the incidence in F1 males was not significantly different from that in the control group, the severity of this lesion was elevated in this dose group (data not shown). In the thyroids, a significantly increased incidence (83-100% of animals) of follicular cell hypertrophy was observed in animals of both sexes in both generations. In the adrenals, a significant increase in the incidence of cortical cell hypertrophy was observed in animals of both sexes in both generations (50% of the males and 33% of the females in F0 generation; 88% of the males and 67% of the females in F1 generation).

No treatment related abnormalities were observed in any of the reproductive organs and pituitary in the 2500 ppm group.

(2) Offspring

2-a Clinical signs and mortality: (data not shown)

The incidence of small body size was relatively higher (4-8% of animals) in F1 pups of the 250 and 2500 ppm groups but this increased incidence was not observed in F2 pups from these dose groups. In the 2500 ppm group, F1 pups from a few litters showed hypothermia, while no F2 pups showed this clinical sign. No treatment-related mortality was observed in offspring.

2-b Mean number of pups delivered, sex ratio, and viability index: (Table 4 attached)

No compound-related adverse effects were observed in the number of pups delivered.

No compound-related adverse effects were observed in the sex ratios with the exception of a significantly lower value (0.473) found in the F2 pups of the 250 ppm group compared to controls (0.558).

No compound-related adverse effects were observed in the viability index with the exception of a significantly higher value (99.4) found on day 0 of lactation in the F2 pups of the 2500 ppm group compared to controls (95.2).

2-c Body weights: (Table 7 attached)

Body weights of F1 and F2 pups of both sexes in the 25 ppm group were comparable to those in the control group. In the 250 ppm group, body weights of F1 pups of both sexes were lower (9-13%), nonstatistically significant, than controls throughout the lactation period with the exception of a significantly lower value (9%) found in male pups on day 0 of lactation. In F2 pups of the 250 ppm group, however, the values of both sexes were comparable to those in the control group. In the 2500 ppm group, body weights of F1 and F2 pups of both sexes were significantly decreased during the most part of the lactation period (9-21% lower compared to controls in F0 generation; 7-19% lower compared to controls in F1 generation).

2-d Gross pathological findings:

In F1 pups found dead during lactation days 0-4 or killed on day 4, body size was significantly increased (7% of animals) in males of the 250 ppm group but this increased incidence was not observed in the 2500 ppm group. White spots located in the periphery of each lobe of the liver and frequently observed in the median lobe were observed in both sexes. The incidence of this change increased with increasing dose and was statistically significant in males (9% of animals) of the 250 ppm group and in both males (36% of animals) and females (40% of animals) of the 2500 ppm group. A slight, but nonstatistically significant increase in the incidence of livers with white spots, was also noted in F1 male pups (7% of animals) of the 25 ppm group. Selected gross hepatic lesions observed in offspring are shown in Table 8.

In F1 pups found dead during lactation days 5-21 or killed at/after weaning, the incidence of white spots in the liver was significantly increased in both males (18% of animals) and females (17% of animals) of the 2500 ppm group. However, these changes were hardly observed in the lower dose groups. A significantly higher incidence (44-51% of animals) of liver enlargement was also detected in both sexes in the 2500 ppm group (data not shown). This liver enlargement correlated histopathologically with diffuse hepatocellular swelling [see section 2) histopathological findings on the liver described below].

In F2 pups found dead during lactation days 0-4 or killed on day 4, white spots in the liver were also observed. The incidence in males (10% of animals) of the 250 ppm group and in both males (50% of animals) and females (44% of animals) of the 2500 ppm group were significantly higher than controls (0% in males; 2% in females). White spots in the

liver were also observed in one female in the 25 ppm and control groups.

In F2 pups found dead during lactation days 5-21 or killed at/after weaning, autopsy revealed that the incidence of white spots was significantly increased in males (20% of animals) and females (14% of animals) of the 2500 ppm group. A significantly higher incidence (43-44% of animals) of liver enlargement was also detected in both sexes in the 2500 ppm group (data not shown).

2-e Histopathological findings on the liver: (Table 9 attached)

In the livers of male and female F1 weanlings autopsied during lactation days 21-27, no adverse effects were observed in the 25 ppm group. Diffuse hepatocellular swelling was detected in male and female F1 weanlings examined in the 250 and 2500 ppm groups. In addition, weanlings of the 2500 ppm group frequently exhibited focal fibrosis/mineralization which may have been preceded by focal hepatocellular necrosis. Bile ductal proliferation and bile stasis were also occasionally observed in these weanlings. [Note: livers of the F1 pups were not available for histopathological examination.]

In F2 pups autopsied during lactation days 0-4, a significantly higher incidence of focal hepatocellular necrosis was observed in both males (78% of animals) and females (85% of animals) of the 2500 ppm group. In the 2500 ppm pups, the incidence of focal fibrosis was significantly higher in male pups (78% of animals) than controls. Such lesions were also noted in female pups in the 2500 ppm group, but the incidence was not statistically significant.

In F2 pups autopsied during lactation days 5-27, diffuse hepatocellular swelling was observed in almost all of the animals examined in the 250 and 2500 ppm groups. In the 2500 ppm group, focal hepatocellular necrosis was found only in one male and one female, while the incidence of fibrosis and mineralization was significantly higher in both males (67% of animals) and females (63-69% of animals) as compared with those in the control group.

V. DISCUSSION AND CONCLUSION

Parental Toxicity: Significant compound-related reductions in body weights and body weight gain were observed in both F1 males and females of the 250 ppm group and in F0 and F1 males and F1 females of the 2500 ppm group. Food consumption was decreased in F1 females of the 250 and 2500 ppm groups. Gross postmortem

examination of parental animals revealed enlargement and discoloration of the liver in virtually all 2500 ppm groups (both generations, both sexes). Relative liver weights increases were observed in F0 males, F0 and F1 females of the 250 ppm group. The absolute and relative liver weights increases were observed in all 2500 ppm groups (both generations, both sexes). Kidney weights increases were observed in F0 females of the 250 ppm group. The absolute and relative kidney weights increases were observed in 2500 ppm group (F0 generation, both sexes). Histopathological examinations revealed hepatocellular swelling (in all high dose group, both generations, both sexes), bile stasis (in F0 and F1 high dose males and F1 high dose females), focal hepatocellular necrosis (in F0 high dose males), focal renal tubular atrophy (in F0 high dose males and females and F1 high dose females), thyroid follicular cell hypertrophy (in F0 and F1 high dose males and females), and adrenal cortical cell hypertrophy (in F0 and F1 high dose males and females and F0 mid dose females).

No compound-related adverse effects were observed on reproductive parameters such as mating, fertility, gestation indices, and length of gestation in F0 and F1 parental animals.

Offspring: Body weights of F1 and F2 pups of both sexes in the high dose group during most part of the lactation period were decreased as compared to controls. Gross examination revealed liver enlargement in F1 and F2 high dose male and female weanlings which was accompanied by histopathological evidence of diffuse hepatocellular swelling in mid dose group (both sexes, F1 and F2 pups) and high dose group (both sexes, both generations). Additional liver toxicity observed was low to high incidence of 'white spots' located on the outer margins of livers of F1 and F2 pups culled on day 4 of lactation at the lowest dietary level of 25 ppm. These spots have been identified histopathologically as localized areas of fibrosis and mineralization and were observed with increasing incidence at all treatment levels. However, liver lesions observed in the low dose F1 pups were not considered to be a treatment related effect based on the absence of statistical significance when compared to control incidence and the fact that this effect was not observed in low dose F2 pups. The lower incidence of fibrosis and mineralization in weanlings (compared to culls) and its complete absence in F1 parental animals indicates that it is fully reversible. There were no indications of treatment related effects on mean number of pups/litter, sex ratio or pup viability in both generations.

The NOEL for parental toxicity is 25 ppm (1.7 mg/kg/day in males; 1.91 mg/kg/day in females). The LEL is 250 ppm (16.4 mg/kg/day in males; 18.6 mg/kg/day in females) based on decreased body weight gain (F1 males), increased relative liver weight (F0 males

and F1 females), and increased absolute kidney weight (F0 females) in parental animals.

The NOEL for reproductive toxicity was equal to or greater than 2500 ppm, the highest dose tested.

MON7200/repro/rats/chin\a:repro.720/Mar/6/91

F0 generation		F1 generation	
Treatment week			
	Quarantine and Acclimatization		
	Assignment 24 males and 24 females (5 weeks of age) per dose level		
1	F0 dosing begins pre-mating period		
11	Determination of estrous cycle		
12	F0 breeding begins Gestation Lactation	Treatment week	
18	Weaning of F1 offspring		Selection 24 males and 24 females per dose level
19	Gross necropsy of F1 offspring		
19	F0 dosing ends Pathological examination of F0 parental animals Gross necropsy Organ weight (10 males and 10 females per dose level) Histopathology	1	F1 dosing begins pre-mating period
		11	Determination of estrous cycle
		12	F1 breeding begins Gestation Lactation
		18	Weaning of F2 offspring
		19	Gross necropsy of F2 offspring
		19	F1 dosing ends Pathological examination of F1 parental animals Gross necropsy Organ weight (10 males and 10 females per dose level) Histopathology

Figure 1. Outline of Reproduction Study with MON 7200 in Rats

Source: Study No. IET 87-0053/ ET-88-3

TABLE 1. Summary of Body Weights (g) for Rats Fed MON 7200 for Two Successive Generations[a]

Dietary Concentration (ppm)	Mean Body Weight at Week			Gestation day		Lactation day	
	1	11	19	7	20	7	21
F0 Males							
0	202	509	585	----------			
25	201	502	569	----------			
250	204	499	571	----------			
2500	207*	484*	537**	----------			
F1 Males							
0	106	506	598	----------			
25	106	500	596	----------			
250	98	470*	564	----------			
2500	94*	454**	532**	----------			
F0 Females							
0	156	297	327	328	433	356	346
25	157	303	328	333	439	361	347
250	158	299	329	328	436	360	357
2500	162**	291	321	315	425	340	348
F1 Females							
0	97	298	330	321	428	364	335
25	95	296	328	315	420	358	332
250	88	293	330	314	420	363	332
2500	81**	266**	296*	289**	396	333*	317

[a] Data were extracted from study No. IET 87-0053/ ET-88-3, Tables 5-8.

TABLE 2. Summary of Body Weight Gain (g) for Rats Fed MON 7200 for Two Successive Generations[a]

Dietary Concentration (ppm)	Mean Body Weight Gain at Week 0-1	0-11	0-19
F0 Males			
0	60	368	444
25	59	360	428
250	63	357	429
2500	66**	343*	396**
F1 Males			
0	44	443	535
25	42	436	531
250	40*	412*	506
2500	39*	399**	477**
F0 Females			
0	36	177	207
25	36	187	208
250	38	179	209
2500	42**	171	201
F1 Females			
0	37	238	271
25	36	237	269
250	34	239	276
2500	32**	217	247

[a] Data were extracted from study No. IET 87-0053/ ET-88-3, Tables 9-12.

TABLE 3. Summary of Food Consumption (g/rat/day) for Rats Fed MON 7200 for Two Successive Generations[a]

Dietary Concentration (ppm)	Mean Food Consumption at Week 1	4	11	Lactation day 0-7	7-14	14-21
F0 Males						
0	21	25	24	----------		
25	21	26*	25	----------		
250	20	25	24	----------		
2500	20	25	26*	----------		
F1 Males						
0	13	25	27	----------		
25	13	25	27	----------		
250	12	23*	26	----------		
2500	12*	25	28	----------		
F0 Females						
0	16	18	17	38	56	71
25	17	18	19*	38	57	71
250	17	18	18	40	55	68
2500	17	18	18	37	50	64
F1 Females						
0	12	19	20	41	61	72
25	12	19	20	41	59	71
250	11**	18*	20	41	58	70
2500	10**	18*	19*	41	55**	65**

[a] Data were extracted from study No. IET 87-0053/ ET-88-3, Tables 13-16.

TABLE 4. Reproductive Data on Parental Animals

GENERATION	DIETARY LEVEL (PPM)	MATING INDEX				FERTILITY INDEX		GESTATION INDEX		DURATION OF GESTATION	MEAN NO. OF PUPS DELIVERED	SEX RATIO	VIABILITY INDEX ON LACTATION DAY		
		MALE		FEMALE									0	4	21
		FRACTION	%	FRACTION	%	FRACTION	%	FRACTION	%				%	%	%
F0	0	23/24	95.8	23/24	95.8	23/23	100.0	23/23	100.0	22.2	13.7	0.505	95.6	91.9	84.1
	25	22/22	100.0	24/24	100.0	24/24	100.0	24/24	100.0	22.3	14.0	0.475	98.4	91.3	79.2
	250	24/24	100.0	24/24	100.0	24/24	100.0	24/24	100.0	22.3	14.6	0.536	97.2	97.9	84.9
	2500	23/23	100.0	24/24	100.0	22/23	95.7	22/22	100.0	22.1	14.6	0.502	91.3	87.1	72.4
F1	0	24/24	100.0	24/24	100.0	21/24	87.5	20/21	95.2	22.2	13.4	0.558	95.2	99.0	100.0
	25	24/24	100.0	24/24	100.0	21/24	87.5	21/21	100.0	22.2	13.2	0.498	98.0	99.3	100.0
	250	23/23	100.0	23/23	100.0	19/23	82.6	18/19	94.7	22.2	13.2	0.473*	97.9	98.8	100.0
	2500	23/24	95.8	23/24	95.8	22/23	95.7	21/22	95.5	22.1	13.2	0.498	99.4*	94.3	99.4

MATING INDEX = (NO. OF COPULATIONS/NO. OF MALES OR FEMALES USED)X100
FERTILITY INDEX = (NO. OF PREGNANCIES/NO. OF COPULATIONS)X100
GESTATION INDEX = (NO. OF NORMAL PARTURITIONS/NO. OF PREGNANCIES)X100
SEX RATIO = TOTAL NO. OF MALE PUPS/TOTAL NO. OF PUPS DELIVERED
VIABILITY INDEX IS GIVEN AS MEAN OF VALUES FROM EACH LITTER BY BEING CALCULATED FROM
VIABILITY INDEX ON DAY 0 = (NO. OF PUPS ALIVE ON DAY 0/NO. OF PUPS DELIVERED)X100
VIABILITY INDEX ON DAY 4 = (NO. OF PUPS ALIVE ON DAY 4/NO. OF PUPS ALIVE ON DAY 0)X100
VIABILITY INDEX ON DAY 21 = (NO. OF PUPS ALIVE ON DAY 21/NO. OF PUPS SELECTED ON DAY 4)X100
* SIGNIFICANTLY DIFFERENT FROM CONTROL AT P<0.05.
** SIGNIFICANTLY DIFFERENT FROM CONTROL AT P<0.01.

Source: Study report IET 87-0053/ET-883, Table 18.

TABLE 5. Summary of Absolute (g) and Relative Organ Weights for Rats Fed MON 7200 for Two Successive Generations[a]

Dietary Concentration (ppm)		Liver	Kidney
Absolute weight			
F0 Males	0	19.3	1.9
	25	18.7	1.9
	250	21.7	2.0
	2500	30.9**	2.1**
F1 Males	0	20.6	1.8
	25	21.3	1.9
	250	20.5	1.7
	2500	33.9**	2.0
Relative weight			
F0 Males	0	32.5	3.1
	25	32.8	3.2
	250	36.4**	3.3
	2500	58.4**	4.0**
F1 Males	0	33.8	3.0
	25	35.2	3.1
	250	36.2	3.1
	2500	64.1**	3.8**
Absolute weight			
F0 Females	0	11.3	1.1
	25	11.8	1.2
	250	13.4	1.2**
	2500	19.0**	1.3**
F1 Females	0	12.9	1.2
	25	12.8	1.2
	250	14.6	1.2
	2500	20.6**	1.2
Relative weight			
F0 Females	0	35.1	3.4
	25	36.8	3.7
	250	40.2	3.7
	2500	60.8**	4.1**
F1 Females	0	40.8	3.7
	25	40.6	3.8
	250	45.6**	3.7
	2500	67.7**	4.0

[a] Data were extracted from study No. IET 87-0053/ ET-88-3, Tables 23-26.
Relative organ weight = mg organ weight / g body weight.
* and ** : Significantly different from the control at 5 and 10% levels of probability, respectively.

TABLE 6. Summary of Incidence of Histopathological Findings for Rats Fed MON 7200 for Two Successive Generations[a]

Dietary Concentration (ppm)		Liver			Kidney	Thyroid	Adrenal
		A	B	C	D	E	F
F0 Males	0	0	0	0	11	0	5
	25	0	0	0	12	0	6
	250	1	0	1	13	0	11
	2500	24**	6*	6*	23**	24**	12*
F1 Males	0	0	0	0	20	0	9
	25	0	0	0	19	0	11
	250	0	0	0	17	0	14
	2500	24**	2	20**	22	23**	21**
F0 Females	0	0	1	0	2	0	1
	25	0	0	0	5	0	2
	250	0	3	0	5	0	8**
	2500	23**	1	3	10**	20**	8*
F1 Females	0	0	2	0	8	0	5
	25	0	1	0	10	0	5
	250	0	2	0	8	0	9
	2500	24**	1	21**	19**	24**	16**

Numbers reflect the findings incidence per group of 24 animals

[a] Data were extracted from study No. IET 87-0053/ ET-88-3, Tables 27-30.

A: Diffuse Hepatocellular swelling
B: Focal Hepatocellular necrosis
C: Bile stasis
D: Focal tubular atrophy
E: Follicular cell hypertrophy
F: Cortical cell hypertrophy

* and ** : Significantly different from the control at 5 and 10% levels of probability, respectively.

TABLE 7. Group Mean Body Weights (g) of F1 and F2 Pups

GROUP MEAN BODY WEIGHTS (G) OF F1 PUPS. MON 7200

LITTER	DIETARY LEVEL (PPM)	MALE PUP WEIGHT ON LACTATION DAY					FEMALE PUP WEIGHT ON LACTATION DAY				
		0	4	7	14	21	0	4	7	14	21
F1 PUP	0	6.8 0.4 23	9.3 1.9 22	13.8 3.3 21	30.7 4.6 19	52.3 6.7 19	6.4 0.4 23	8.8 1.8 22	13.3 3.3 21	29.2 4.7 19	49.6 7.1 19
	25	6.7 0.5 24	8.5 1.8 24	13.3 3.4 22	29.8 5.5 20	51.3 8.5 20	6.4 0.4 24	8.3 1.7 23	12.8 3.6 22	28.3 6.4 20	48.0 9.3 20
	250	6.4* 0.4 24	8.5 1.4 24	12.0 3.3 24	27.0 5.1 21	48.1 6.4 21	6.1 0.5 24	8.2 1.4 24	11.7 3.1 24	25.9 4.6 22	45.4 6.5 22
	2500	6.1** 0.5 22	7.8* 1.8 21	12.5 1.6 16	25.4* 3.5 16	43.8** 4.3 15	5.7** 0.4 22	7.4* 1.5 22	11.0 2.0 18	23.0** 3.8 17	39.7** 4.3 16

VALUES REPRESENT MEAN, S.D. AND NO. OF LITTERS EXAMINED.
* AND **: SIGNIFICANTLY DIFFERENT FROM THE CONTROL AT 5 AND 1% LEVELS OF PROBABILITY, RESPECTIVELY.

GROUP MEAN BODY WEIGHTS (G) OF F2 PUPS. MON 7200

LITTER	DIETARY LEVEL (PPM)	MALE PUP WEIGHT ON LACTATION DAY					FEMALE PUP WEIGHT ON LACTATION DAY				
		0	4	7	14	21	0	4	7	14	21
F2 PUP	0	6.7 0.4 20	11.3 1.1 20	17.7 2.1 20	36.1 3.9 20	60.1 5.8 20	6.3 0.4 20	10.6 1.0 20	16.6 1.8 20	34.4 3.6 20	56.5 5.1 20
	25	6.6 0.7 21	11.2 2.1 21	17.6 2.9 21	36.1 4.4 21	60.6 6.9 21	6.3 0.7 21	10.8 2.0 21	16.8 2.6 21	34.4 3.9 21	57.2 5.8 21
	250	6.5 0.4 18	11.1 1.3 18	17.2 2.2 18	34.6 3.7 18	57.7 5.5 18	6.2 0.5 18	10.6 1.3 18	16.7 2.1 18	33.5 3.4 18	54.3 5.2 18
	2500	6.2** 0.4 20	9.8** 1.1 20	15.4* 1.7 20	30.2** 3.0 20	48.6** 4.7 20	5.9 0.5 21	9.3** 1.1 20	14.8 1.7 20	28.9** 2.6 20	46.0** 4.0 20

VALUES REPRESENT MEAN, S.D. AND NO. OF LITTERS EXAMINED.
* AND **: SIGNIFICANTLY DIFFERENT FROM THE CONTROL AT 5 AND 1% LEVELS OF PROBABILITY, RESPECTIVELY.

Source: Study Report IET 87-0053/ET-88-3, Table2 35 and 36.

TABLE 8. Summary Incidence of Selected Gross Hepatic Lesions Observed in Offspring on Two Generation Study with MON 7200

		Incidence[a] of Pups with Liver White Spots[b]			
Dose Level (ppm)		0	25	250	2500
F1 pups examined on lactation day 4 [c]	♂	0/58	4/61	8/89*	26/71**
	♀	0/59	1/73	2/63	24/60**
F1 pups examined at weaning (days 21-27) [d]	♂	0/54	0/50	1/62	7/40**
	♀	0/48	0/55	0/59	6/41**
F2 pups examined on lactation day 4 [c]	♂	0/63	0/54	4/41*	31/61**
	♀	1/42	1/59	5/49	28/60**
F2 pups examined at weaning (days 21-27) [d]	♂	0/84	1/83	0/69	16/79**
	♀	0/75	0/78	0/75	11/80**

* - $p \leq 0.05$, ** - $p \leq 0.01$, Fisher's Exact Test

a - Incidences represent (number of pups with lesion/number of pups examined).

b - Liver white spots have been identified histopathologically as localized areas of fibrosis and mineralization.

c - Includes pups found dead between lactation days 0-4.

d - Includes pups found dead between lactation days 5-21.

TABLE 9. Incidence of Liver Histopathological Findings in F1 and F2 Pups On Two Generations Study with MON 7200 [a]

Findings	Dietary Concentration (ppm)			
	0	25	250	2500
Male F1 Pups autopsied during lactation days 21-27				
No. of pups examined	5	5	6	12
Diffuse hepatocellular swelling	0	0	6**	12**
Fibrosis/Mineralization	0	0	0	5
Female F1 Pups autopsied during lactation days 21-27				
No. of pups examined	5	5	5	10
Diffuse hepatocellular swelling	0	0	5**	10**
Fibrosis/Mineralization	0	0	0	3
Male F2 Pups autopsied during lactation days 0-4				
No. of pups examined	5	5	9	36
Diffuse hepatocellular swelling	0	0	4	28**
Fibrosis	0	0	0	28**
Mineralization	0	0	0	2
Female F2 Pups autopsied during lactation days 0-4				
No. of pups examined	6	6	10	33
Diffuse hepatocellular swelling	1	1	4	28**
Fibrosis	1	2	2	17
Mineralization	0	0	0	1
Male F2 Pups autopsied during lactation days 5-27				
No. of pups examined	5	6	5	21
Diffuse hepatocellular swelling	0	0	5**	21**
Fibrosis	0	1	0	14*
Mineralization	0	1	0	14*
Female F2 Pups autopsied during lactation days 5-27				
No. of pups examined	5	5	5	16
Diffuse hepatocellular swelling	0	0	4*	16**
Fibrosis	0	0	0	11*
Mineralization	0	0	0	10*

[a] Data were extracted from study No. IET 87-0053/ ET-88-3, Tables 27-30.

* and ** : Significantly different from the control at 5 and 10% levels of probability, respectively.