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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review a reproduction study with Thiobencarb
EPA ID No. 239-2431 Tox Proj No. 8-0428
EPA Acc No. 404462-01 Caswell No. 297 DA

FROM: Quang Q. Bui, Ph.D. Quang Q Bui 4/1/88
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TO: Richard Mountfort, PM # 23
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THRU: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Chevron Chemical Co.
Richmond, California 94804

Action Requested: Review a multigeneration reproduction study with Thiobencarb in rats - CIEA No. ML-289A2, dated 12/7/87.

BACKGROUND INFORMATION:

A reproduction study in rats (Biodynamics #82-2615) was previously submitted to the Agency under Accession Nos. 256017-256022. The study was reviewed and classified as Core Supplementary Data (memo of Q. Bui to R. Mountfort, 7/12/85) on account of various deficiencies, including reduced pregnancy rate and male fertility indices in control males.

In this action, a repeat reproduction study is submitted to fulfill the registration requirement for a 2-generation rat reproduction study under 40 CFR 158.135, Pesticide Assessment Guideline No. 83-4.

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DATA EVALUATION RECORD

Chemical: Thiobencarb - Bolero
Test Material: Technical 96.7% a.i.
Study Type: Reproduction study

STUDY IDENTIFICATION

Reproduction Study by Oral Forced Administration of Thiobencarb in Rats - Main Experiment

Testing Facility: Central Inst. for Experimental Animals Kawasaki, Japan
Final Report No.: CIEA No. ML-289A2
Final Report Date: 12/7/87
Study Authors: Y. Hatakeyama and T. Yanagita

EPA Acc. Nos.: 404462 -01/02
Study Reviewed by: Quang Q. Bui, PhD., DABT. Quang Q Bui 4/1/88
Head, Review Section V
Toxicology Branch/HED (TS-769C)

RECOMMENDATION:

Under the conditions of this investigation, the systemic NOEL is established at less than 2 mg/kg (LDT). Findings of liver weight changes, enlargement of centrolobular hepatocytes, and renal atrophic tubules were noted at higher dosage levels. Significant decreases in body weight were found at the 100 mg/kg dose level in F0 and F1 males during the premating period and in F1 females during the premating, gestation, and lactation periods.

Administration of Bolero at 2, 20, or 100 mg/kg did not affect the reproductive performance of both males and females throughout two consecutive generations. However, decreases in the viability index (day 4 offspring survivability) and pup weights were noted in high dose F1 offspring (born from F0 parental animals) during the lactation period. Maturational landmarks recorded postnatally were not affected except for a delay in eye opening observed in F2 offspring at the 100 mg/kg dose level. The biological significance of this finding is not known with certainty and requires an explanation from the study authors (see Discussion section).

It is recommended that this study be classified as Core Minimum Data. The registrant has fulfilled the regulatory requirements for a multigeneration reproduction study. The systemic NOEL is established at less than 2 mg/kg. The reproductive NOEL is tentatively established at 20 mg/kg. This reproductive NOEL may be re-established at a higher dosage level pending the submission of additional information relative to the procedures for assessing maturational landmarks (see Discussion section).

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MATERIALS AND METHODS:

Test Material:	Technical Bolero, Lot L 6006
Purity:	96.7 % a.i.
Solvent:	0.5% CMC
Animals:	Jcl:SD Rats
Source:	Nihon Clea Co., Japan
Initial Weight:	173-198 g (males) 137-170 g (females)
Dose levels:	0.5% CMC (control), 2, 20, and 100 mg/kg
Dose volume:	10 ml/kg
Route of adminis.:	Gavage

In general, the protocol used was designed after Sect 83-4 of the 1982 FIFRA Guidelines and, therefore, is acceptable. The following deviations were noted:

1. In cases that mating within the same dose group did not occur within 2 weeks, males and females were re-mated to untreated animals of the opposite sex. Males which failed to impregnate a female or females which failed to become pregnant were also re-mated to untreated animals of the opposite sex.

2. Histopathologic examinations were conducted on all F0 and F1 adults of all groups.

3. Daily observation of the estrous cycle was performed on all F0 and F1 females prior to the mating period.

4. A live born index was presented by the authors and was defined as the ratio of live pups/number of implantations. The post-implantation loss was not calculated.

5. Apparently, necropsy was not performed on F1 weanings not selected as parental animals and on F2 offspring.

RESULTS:

ANALYTICAL DETERMINATION:

Technical Thiobencarb was prepared weekly at concentrations of 0.02, 0.2, and 1.0% w/v in 0.5% CMC suspension. Concentrations of Thiobencarb were calculated based upon a specific gravity of 1.169 and a purity of 96.7%. Analytical determinations were conducted throughout the study and all values were within 10% of the nominal concentrations.

STABILITY DATA

After one week, the concentration of Thiobencarb in the 0.5% CMC suspension was 99.0, 96.0, and 99.0% for the 0.2, 2, and 10 mg/ml dosing solutions, respectively.

CLINICAL OBSERVATIONS:

F0 Generation: Adults
One female in the 20 mg/kg group died (#P 703). Changes attributable to treatment were not found.
F1 Generation: Adults
One female each in the 20 (# S708) and 100 mg/kg (S 817) groups died on lactation day 1 and 5, respectively. One control female (# S503) died on lactation day 18 due to handling accident. No treatment-related clinical signs were found among all groups.

BODY WEIGHTS

F0-Premating Period:
During the premating period (weeks 1-12), significant decreases in body weights were noted in 100 mg/kg males but not in females.

F0-Female Gestation Period:
No statistically significant differences in absolute body weights or body weight gains were found among all groups.

F0-Female Lactation Period:
No statistically significant differences in absolute body weights or body weight gains were found among all groups.

F1-Premating Period:
Statistically significant decreases in body weights were noted in 100 mg/kg males throughout the entire premating period (weeks 1-14). The high dose female body weights were also statistically decreased from control values from weeks 9-14.

F1-Female Gestation Period:
Throughout gestation, a statistically difference in body weight was found only in 100 mg/kg females.

F1-Female Lactation Period:
The significant decrease in body weight found in 100 mg/kg females persisted during the lactation period up to post-natal day 21.

FOOD CONSUMPTION:
F0 Parental Animals:
During the premating period, food intake was significantly decreased in low- and mid-dose males but was significantly increased in high-dose males from weeks 8 to 11. In females, significant increases in food intake were found in mid- and high-dose groups. Sporadic changes in food intake were noted in treated females during the gestation and lactation periods.

F1 Parental Animals:
High dose males had significantly higher food intake than controls during the premating period. Significant decreases were noted in low- and mid-dose females but significant increases in food intake were observed with high-dose females. Reduced food

intake was noted in all treated females during the gestation period.

REPRODUCTIVE PERFORMANCE - MALES

	0 MG/KG	2 MG/KG	20 MG/KG	100 MG/KG
F0 GENERATION				
No. male rats used	25	25	24	25
No. males copulated	25	23	22	25
Male Mating Index	100%	92%	92%	100%
No. males impregnated at least 1 female	24	16	20	20
Male Fertility Index[a]	96%	70%	91%	80%
No. males remated	1	9	5	5
No. males copulated	1	9	5	5
No. males impregnated	1	9	4	4
Total males copulated	25	25	25	25
Total males impregnated	25	25	24	24
Overall Male Fert.Index	100%	100%	96	96
F1-GENERATION				
No. males used	25	25	25	25
No. males copulated	21	20	25	25
Male Mating Index	84%	80%	100%	100%
No. males impregnated at least 1 female	13	16	21	20
Male Fertility Index	62%	80%	84%	80%
No. males remated	12	9	4	5
No. males copulated	12	8	4	5
No. males impregnated	12	7	4	3
Total males impregnated	25	23	25	23
Overall Male Fert.Index	100%	92%	100%	92%

(a) Male fertility index = Males impregnated/Males copulated

In the F0 generation, a slight decrease in the male fertility index was noted in low (70%) and high dose (80%) groups. However, when those animals, which had failed to copulate or impregnate a female, were remated to untreated females, no differences in the Overall Male Fertility Index were found suggesting that the decreases noted could possibly be female-mediated.

In the F1 generation, the male fertility index was lowest in the control group (62%) as compared to 80% of the treated groups. After remating, the Overall Male Fertility Index for all groups was acceptable ranging from 92 to 100%.

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FEMALE REPRODUCTIVE PERFORMANCE - FEMALES

	0 MG/KG	2 MG/KG	20 MG/KG	100 MG/KG
F0 GENERATION				
No. females used	25	25	24	25
No. females copulated	25	25	24	25
Mating Index @	100%	100%	100%	100%
No. females pregnant	24	17	21	20
Fertility Index[a]	96%	68%	88%	80%
No. females remated	1	8	3	5
No. females pregnant	0	2	3	3
Total No. pregnant	24	19	24	23
Overall Fertility Index	96%	76%	100%	92%
Mean gestation length (d)	22.5	22.3	22.3	22.4
F1 GENERATION				
No. females used	25	25	25	25
No. females copulated	24	22	25	25
Mating Index	96%	88%	100%	100%
No. females pregnant	16	18	21	20
Fertility Index	67%	82%	84%	80%
No. females remated	9	7	4	5
No. females pregnant	6	4	2	2
Total No. pregnant	22	22	23	22
Overall Fertility Index	88%	88%	92%	88%
Mean length gestation (d)	22.3	22.1	22.4	22.4

(@) Mating index = No. copulated/No. exposed
(a) Fertility Index = No. pregnant/No. copulated

In the F0 generation, the female fertility index calculated from mating within group was low for the 2 mg/kg group, being 76%. When these animals were remated with untreated males, a 88% overall fertility index was obtained. These findings suggest that the low fertility index associated with this group may well be related to treatment. However, in the absence of a similar effect in the 20 and 100 mg/kg groups, the findings in the 2 mg/kg group must be regarded as incidental.

REPRODUCTIVE STATUS

	0 MG/KG	2 MG/KG	20 MG/KG	100 MG/KG
F0-Generation				
No. dams pregnant	24	17	21	20
No. dams delivered	24	17	21	19
Mean Implantations/dam	13.3	14.9	13.1	14.4
Mean Litter Size	11.5	13.0	11.7	11.5
Mean dead pup/dam	0.5	0.5	0.2	0.5
Live born rate [a]	83.8%	87.7%	86.5%	79.4%
Male pup born weight (g)	6.6	6.3	6.6	6.2
Female pup born weight	6.2	6.1	6.6	6.0
F1-Generation				
No. dams pregnant	16	18	21	20
No. dams delivered	15	18	21	20
Mean implantations/dam	15.1	14.4	13.6	13.0
Mean litter size	12.3	13.1	12.2	11.4
Mean dead pup/dam	1.6	0.2	0.2	0.5
Live born rate [a]	79.0%	90.9%	90.3%	90.8%
Male pup born weight (g)	6.3	6.5	6.5	6.5
Female pup born weight	6.0	6.1	6.0	6.0

(a) Live born rate = No. live born pups/No. of implantations

In both generations, no differences in mean implantations, mean litter size, and mean dead pups per dam were found among all groups. The live born rate for both generations was adequate. No statistically significant differences in pup weights at birth were found.

OFFSPRING DATA

As indicated in the table on the next page, the viability index (day 4 survival) calculated for the F1 offspring was significantly decreased at the 100 mg/kg dosage level. No differences in the weaning index (day 21 survival), were found. However, it should be noted that all litters were culled to 8 pups on day 4 of lactation. Had the litters not been culled, differences in this index would possibly be obtained. Differences in either viability or weaning index were not found in F2 offspring.

OFFSPRING DATA

	0 MG/KG	2 MG/KG	20 MG/KG	100 MG/KG
F1-OFFSPRING				
Viability Index	97.9%	98.8%	94.0%	93.3%*
Weaning Index	98.6%	100.0%	99.4%	97.4%
Weight day 4 (g)				
Males	10.1	10.0	10.5	9.2
Females	9.8	9.8	9.7	8.6*
Weight day 4 (after culling)				
Males	10.0	10.1	10.5	9.2
Females	9.8	9.9	9.8	8.7*
Weight day 7				
Males	16.4	16.9	17.1	15.0*
Females	15.6	16.5	15.9	14.2
Weight day 21				
Males	56.3	56.8	57.1	52.5
Females	54.3	56.0	53.6	50.3
F2-OFFSPRING				
Viability Index	92.4%	92.9%	96.9%	95.7%
Weaning Index	99.1%	99.3%	100.0%	98.0%
Weight day 4 (g)				
Males	10.0	9.8	10.3	9.8
Females	9.5	9.3	9.8	9.4
Weight day 7				
Males	16.8	16.6	17.0	16.0
Females	15.8	15.5	16.0	15.4
Weight day 21				
Males	59.2	57.4	58.2	55.1
Females	56.3	54.1	55.4	53.3

(*) Significantly different from controls, $P < 0.05$

Although no differences in pup weights were found in both generations at birth, a statistically decrease in weight was found in 100 mg/kg females on postnatal day 4 and 100 mg/kg males on postnatal day 7 of the F1 generation. Similar effects on pup weights were not observed in the F2 generation.

OFFSPRING MATURATIONAL LANDMARKS

In addition to body weights, several maturational landmarks were also monitored by the investigators.

Respective standardized times (in days) to pinna detachment, hair growth, tooth eruption, opening of auditory canals, and eye opening were 4, 11, 12, 14, and 15.

In F1 offspring, no differences in maturational landmarks were observed between the control and treated groups.

In F2 offspring, a statistically significant delay in time to eye opening was observed in the 100 mg/kg group with 95.2% of pups had their eyes opened by day 15 of lactation as compared to 100% of controls. The biological significance of this finding is unclear since the procedures for assessing this parameter are not described in the final report.

PARENTAL ORGAN WEIGHTS

Organ weight data are available for parental animals of both generations. Only findings of interest are discussed in this memorandum

F0-generation:

Statistically significant increases in both absolute and relative liver weights were noted in 20 and 100 mg/kg males and females. A statistically significant increase in both absolute and relative kidney weights was found only in 100 mg/kg males. In 100 mg/kg females, a significant decrease in absolute and relative pituitary weights was observed.

F1-generation:

In males, increases in absolute and relative liver weights were found at the 100 mg/kg dosage level. Significant decreases in absolute pituitary weight in mid and high dose groups and in absolute adrenal weight in high dose group were reported. The relative testis weight was significantly increased at the 20 and 100 mg/kg levels but may result from a decrease in terminal body weight associated with these groups.

In females, no differences in absolute liver weights were found but the relative weight of the 100 mg/kg was increased. Significant increases in relative pituitary weights were noted in all groups along with relative adrenal weight in the mid and high dose groups.

HISTOPATHOLOGY

F0 GENERATION-ADULTS

Enlargement of centrolobular hepatocytes was observed in 0/25, 9/25. 25/25, and 25/25 males in the 0, 2, 20, and 100 mg/kg groups and in, respectively, 0/24, 4/17, 6/21. and 19/20 females.

HISTOPATHOLOGY FINDINGS

	0 MG/KG	2 MG/KG	20 MG/KG	100 MG/KG
Enlargement of centrolobular hepatocytes				
F0 males	0/25	9/25	25/25	25/25
F0 females	0/24	4/17	6/21	19/20
F1 males	0/25	0/25	22/25	25/25
F1 females	0/16	0/18	6/21	19/20
Single cell necrosis - hepatocytes				
F0 males	0/25	1/25	1/15	7/25
F0 females	7/24	4/17	8/21	8/20
F1 males	1/25	3/25	7/25	17/25
F1 females	0/16	1/18	4/21	4/20
Kidney, atrophic tubule consisting of regenerated epithelium				
F0 males	0/25	3/25	2/25	24/25
F0 females	0/24	1/17	2/21	0/20
F1 males	3/25	7/25	14/25	24/25
F1 females	0/16	0/18	0/21	0/20

The severity of the enlargement of centrolobular hepatocytes was dose-related. Single cell necrosis of hepatocytes was found in 0/25. 1/25, 1/25, and 7/25 males, respectively. In females, the incidences of single cell hepatocyte necrosis were 7/24, 4/17, 8/21, and 8/20, respectively. Treatment-related increases in kidney, atrophic tubule were found in males but not females.

F1-generation:

Findings of hepatocyte centrolobular enlarged and single cell necrosis observed in the F0 generation were also noted in both males and females of the F1 generation. A treatment-related increase in kidney with atrophic tubule was also found in F1 males.

DISCUSSION:

Oral administration of technical Bolero at 2, 20, or 100 mg/kg/day to male and female rats did not result in significant increase in lethality or overt signs of toxicity. Significant decreases in body weights were noted in F0 and F1 males at the 100 mg/kg dosage level. Although no body weight differences were found in all F0 treated females, a statistical difference was attained in the F1 generation at the 100 ppm dosage level during the premating period, gestation, as well as lactation. The changes in food consumption were sporadic in nature (decreased in low dose but increased in high dose animals) and, hence, are of questionable biological significance.

In the F0 generation, significant increases in absolute and relative liver weights were noted in 20 and 100 mg/kg males and females. The kidney weights were also statistically increased at the 100 mg/kg dosage level for males. In the F1 generation, changes in liver weights were found in 100 mg/kg males but not in females. The pituitary weight was significantly decreased in males of the 20 and 100 mg/kg groups but was significantly increased in all female treated groups. Upon histopathologic examination, dose-related increases in incidence and severity of "enlargement of centrolobular hepatocytes" were found in both generations for both males and females. Higher incidences of "hepatocytes single cell necrosis" were also observed in high dose males and females of both generations and in mid dose males and females of the F1 generation. The incidences of "kidney, atrophic tubule consisting of regenerated epithelium" were increased in F0 high dose and F1 mid and high dose groups.

The investigators claimed that the changes in the liver were due to enzyme induction connected with Bolero. However, it should be noted that from a chronic feeding study with technical Bolero (Life Sciences Ltd., 9/7/84), a statistically significant decrease in liver weight was noted at the 100 ppm dosage level (highest dose tested, approximately 25 mg/kg) in both males and females (memo of Q. Bui to R. Mountfort, 2/15/85). No enlargement of centrolobular hepatocytes were reported in the chronic study. Findings of "kidney, atrophic tubule" were also absent in the chronic feeding study. "Swelling of the centrilobular hepatocytes" and "kidney, tubular degeneration" were also observed in the previous multigeneration reproduction study in rats (Bio/dynamics #82-2615, memo of Q. Bui to R. Mountfort dated 7/12/85) in 10 and 40 mg/kg F1 and F2a males. These pathological changes were thus confirmed in this new reproduction study. Collectively, the findings in the kidney and liver should be considered as treatment related. In the males, the incidences of "kidney, atrophic tubule" in the 0, 2, 20, and 100 mg/kg groups were, respectively, 0, 12, 8, and 96% for the F0 generation, and 12, 28, 56, and 98% for the F1 generation.

Based upon the above findings, the systemic NOEL is established at less than 2 mg/kg. The systemic LEL is 2 mg/kg with increased incidences of renal atrophic tubules found at this dosage level.

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The male fertility index apparently was decreased at the 2 and 100 mg/kg of the F0 generation when mating was conducted with animals of the same treatment group. Upon remating with untreated females, this index became acceptable suggesting that the decreases may be female-mediated. The female fertility index for the F0 generation was low (76%) for the 2 mg/kg group. However, in the absence of a dose response relationship, the finding was treated by this reviewer as incidental. In the F1 generation, both male and female fertility indices were low in the control group, being 62 and 67%, respectively. After remating, both indices were elevated to an acceptable level (100 and 88%, respectively). No significant differences in the means of implantation, litter size, [illegible]ion, live born rate, pup birth weight were noted in both [illegible]ations. In the F1 offspring (pups born from F0 parental animals), the viability index (day 4 survivability) was significantly decreased at the 100 mg/kg dose level, but no differences in the weaning index was found. It should be noted, however, that culling was conducted in this study. Had culling not been performed, a significant decrease in the weaning index at the 100 mg/kg dose level may be achieved. No differences in the viability or weaning indices were found in F2 offspring (pups born from F1 parental animals). In the absence of a consistent effect throughout both generations and due to the magnitude of the decrease in the viability index (93.3% - 100 mg/kg vs. 97.9% - control), this finding was not regarded as treatment related by this reviewer. Transient decreases in pup weights were found only in F1 offspring affecting 100 mg/kg males on postnatal day 7 and 100 mg/kg females on postnatal day 4. No statistical differences were found in offspring weight at weaning. No alterations in weights were noted in F2 offspring from birth up to weaning.

Maturational landmarks as characterized by time to pinna detachment, eye opening, hair growth, tooth eruption, and auditory canal opening were similar between the control and treated pups except for a delay in eye opening noted in 100 mg/kg F2 offspring. The biological significance of this delay is unclear since the procedures for assessing this parameter are not described in the final report. It is unclear to this reviewer as to what would fulfill the criteria of this parameter: Is this parameter met when one eye is fully opened or must both eyes be opened? The same question would also apply for the parameter "auditory canal opening".

Based upon the above findings, the reproductive NOEL is tentatively established at 20 mg/kg. This reproductive NOEL may be re-established at a higher dosage level pending the submission of a detail description of the methods used to assess maturational landmarks.

It is recommended that this study be classified as Core Minimum Data. The registrant has fulfilled the regulatory requirements for a multigeneration reproduction study.

Systemic NOEL < 2 mg/kg Systemic LEL = 2 mg/kg