SECTION HEAD

(37)

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

APR 29 1988

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 3,5,6-Trichloro-2-Pyridinol (TCP), a Metabolite of Chlorpyrifos: One 3-Month Rat Dietary Toxicity Study, A Rat Teratogenicity (Developmental Toxicity) Study, A Rabbit Teratogenicity (Developmental Toxicity) Study and Four Mutagenicity Studies. Caswell No. 821AA; EPA ID 3F2884/1H5295/3H5396; Tox. Proj. No. 8-0213.

FROM: Alan C. Levy, Ph.D. Alan C. Levy 4/28/88
Toxicologist, Review Section V
Toxicology Branch/HED (TS-769C)

TO: Dennis Edwards (PM 12)
Registration Division (TS-767C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q Bui 4/28/88
Acting Section Head, Review Section V

and

Theodore M. Farber, Ph.D., D.A.B.T. 4/29/88
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Dow Chemical Company

Action: Review the 3-month rat dietary toxicity study, the rat teratogenicity (developmental toxicity) study, the rabbit teratogenicity (developmental toxicity) study and four mutagenicity studies.

Recommendations:

1. A THREE-MONTH RAT DIETARY TOXICITY STUDY: This study is classified as CORE MINIMUM.

Systemic Toxicity NOEL = 30 mg/kg/day (mid dose)
Systemic Toxicity LOEL = 100 mg/kg/day (high dose)

The high dose of 100 mg/kg/day appeared to cause an increase in liver and kidney weights.

2. A RAT TERATOGENICITY (DEVELOPMENTAL TOXICITY) STUDY: This study is acceptable and is considered to be CORE MINIMUM.

Maternal Toxicity NOEL = 50 mg/kg/day (low dose)
Maternal Toxicity LOEL = 100 mg/kg/day (mid dose)
Developmental Toxicity NOEL = 150 mg/kg/day (high dose)
Developmental Toxicity LOEL = > 150 mg/kg/day (high dose)

There appeared to be a group mean decrease in body weight gain during dosing (days 6 through 15 of gestation) in the mid- (100 mg/kg/day) and high- (150 mg/kg/day) dose groups. There were no apparent effects on fetal development.

3. A RABBIT TERATOGENICITY (DEVELOPMENTAL TOXICITY) STUDY: This study is classified as SUPPLEMENTAL. This may be upgraded to CORE MINIMUM provided that answers to the artificial insemination questions are acceptable.

Maternal Toxicity NOEL = 100 mg/kg/day (mid dose)
Maternal Toxicity LOEL = 250 mg/kg/day (high dose)
Developmental Toxicity NOEL = 25 mg/kg/day (low dose)
Developmental Toxicity LOEL = 100 mg/kg/day (mid dose)

There was an apparent maternal mean body weight loss in the 250 mg/kg/day group only during the period of dosing (days 7 through 19 of gestation). There was the suggestion of an increase of central nervous system anomalies (hydrocephaly/dilated cerebral ventricles) in both the number of fetuses and litters at doses of 100 and 250 mg/kg/day.

4. MUTAGENICITY STUDIES

A. Ames Salmonella/Mammalian Microsome Mutagenicity Assay: This study is considered ACCEPTABLE. No significant increase in revertant (or mutant) frequency was observed.

B. Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay: This study is considered ACCEPTABLE. No positive UDS response was observed.

C. Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay: The portion of the study "with S9" is considered ACCEPTABLE. The portion of the study "without S9" is considered UNACCEPTABLE because the maximum concentration of 750 ug/ml was not considered to be high enough. Both aspects of the study "with" and "without" S9 were considered to be negative under the conditions of the assay. It is considered that a repeat of the portion "without S9" at higher concentrations is not judged necessary at the present time. Although the 750 ug/ml concentration without S9 did not achieve proper toxicity, there was no suggestion of activity. Also, no survival was seen at 1000 ug/ml. Therefore, to perform an additional experiment at concentrations between these two may not provide additional critical information for assessment at this time.

D. Mouse Bone Marrow Micronucleus Test: This study is considered to be UNACCEPTABLE because the doses tested were not high enough (no signs of toxicity reported). Additional data are required.

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Primary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy 4/27/88
Review Section V/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T. Quang Bui 4/29/88
Acting Section Head, Review Section V

I. Study Type: Teratogenicity Study (Developmental Toxicity) (Guideline § 83-3)

Study Title: 3,5,6-Trichloro-2-Pyridinol: Oral Teratology Study in Fischer 344 Rats

EPA Identification:

EPA Identification: 1H5295
EPA Accession: 403488-02
EPA Record: 205143
Caswell: 821AA
Tox. Branch Project: 8-0213

Sponsor: The Dow Chemical Company
Midland, MI 48674

Testing Laboratory: Mammalian and Environmental Toxicology Research Laboratory
Health and Environmental Sciences
The Dow Chemical Company
Midland, MI 48674

Study Number: K-038278-011

Study Date: July 23, 1987

Study Authors: T. R. Hanley, M.S., D.A.B.T., G. J. Zielke, B.S., and L. G. Lomax, D.V.M., Ph.D., D.A.C.V.P.

Recommendation:

The Maternal Toxicity No Observed Effect Level (NOEL) is 50 mg/kg/day. The Maternal Toxicity Lowest Observed Effect Level (LOEL) is 100 mg/kg/day (mid dose). There appeared to be a group mean decrease in body weight gain during dosing (days 6 through 15 of gestation) in the mid- (100 mg/kg/day) and high- (150 mg/kg/day) dose groups. There were no apparent dosing related effects regarding any other parameters.

The Developmental Toxicity No Observed Effect Level (NOEL) is 150 mg/kg/day (the highest dose tested - HDT). The Developmental Toxicity Lowest Observed Effect Level (LOEL) is >150 mg/kg/day.

The study is acceptable and is considered to be Core Minimum.

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Test Material:

Name: 3,5,6-trichloro-2-pyridinol (TCP)

Lot No.: AGR # 143197

Purity: 99.7%

Vehicle: 0.5% aqueous METHOCEL

NOTE: The material is the major degradation product of chlorpyrifos, the active ingredient in DURSBAN and LORSBAN brand insecticides.

II. Materials and Methods

The test material was suspended in a vehicle of 0.5% aqueous METHOCEL and administered to rats by oral gavage at a volume of 4 ml per kg body weight. The control group received the 0.5% METHOCEL vehicle. Homogeneity and stability were tested with 25 mg/ml of METHOCEL and were found to be satisfactory for 76 days. The test suspension for each dose level was analyzed to determine the concentration of the test material once during the study.

Fischer 344 rats from Charles River Breeding Laboratories, Kingston, NY were used in the study. Adult virgin females (approximately 150-225 g) were bred overnight with males (one male to one female) with Day 0 of gestation being the presence of sperm in vaginal smears. Randomization according to Day 0 of gestation was performed by a computer-generated table of random numbers. Groups of 32-34 bred females were administered TCP by oral gavage on Days 6 through 15 of gestation at doses of 0, 50, 100 and 150 mg/kg/day. [The dose levels were based upon results of a prior probe study.] Body weights were recorded on Day 0, daily on Days 6 through 16 and on Day 21 of gestation. Dose volumes were adjusted daily. Cesarean sections were performed on Day 21. Maternal liver, kidney and gravid uterine weights were recorded. Sections of liver and kidneys were preserved in phosphate-buffered 10% formalin, but no histopathologic examination was performed.

At Cesarean section on day 21 of gestation, the following data were recorded: number and position of fetuses *in utero*; number of live and dead fetuses; number and position of resorption sites; number of corpora lutea; sex and body weight of each fetus; and any gross external alterations. Uteri of apparently non-pregnant rats were stained with a 10% solution of sodium sulfide and examined for evidence of implantation sites. Using a table of random numbers, one-half of each litter was dissected under a low power stereomicroscope and examined for soft tissue changes. The heads of fetuses examined by dissection were removed, placed in Bouin's fixative and examined by the serial sectioning technique of Wilson. All fetuses were then preserved in alcohol, eviscerated, cleared, stained with alizarin red-S and examined for skeletal alterations.

Detailed descriptions of statistical analyses employed were described.

A Quality Assurance statement was included.

A copy of the Materials and Methods section from the report is appended.

There are no comments regarding the Materials and Methods section.

III. Results

Table 1 presents analyses of the dosing suspensions. Liquid chromatography indicated that suspensions used were 103-108% of the desired concentration.

Table 1

SUMMARY OF 3,5,6-TRICHLORO-2-PYRIDINOL DOSE SOLUTION ANALYSES[a]

Targeted Dose (mg/kg/day)	% of Targeted Concentration Mean ± S.D.
Control	ND[b]
50	108±1
100	103±1
150	108±4

a = Mean of 2 injections of 1 aliquot per sample.
b = N.D. - not detected, detection limit 0.9 mg/g METHOCEL suspension.
This table is reproduced from Table 1, page 18 of the report.

There were no clinical signs which appeared to be attributable to compound administration. One control animal was necropsied in a moribund condition. Examination revealed an intubation error as the probable cause of the rat's condition.

A statistically significant decrease in body weight gain was observed during the period of dosing (days 6 through 15) in the mid- (100 mg/kg/day) and high- (150 mg/kg/day) dose groups. (See Tables 2 and 3). Prior to dosing (days 0-6) and post dosing (days 16-21), the body weight gains of these two dose groups were similar to the

control values. There were slight decreases in mean food consumption in the mid- and high-dose groups during dosing, but these were considered to be within the "normal" range.

Table 2

MEAN BODY WEIGHTS (GRAMS) OF PREGNANT RATS ADMINISTERED TCP

Mg/kg/day	Day of Gestation 0	6	9	12	16	21	21(†)
0	180.9[a]	195.3	200.9	209.0	224.4	265.6	208.5
	14.4[b]	10.1	10.2	10.7	12.1	14.2	10.2
	23[c]	30	30	30	30	30	30
50	178.5	193.2	198.7	205.7	218.7	258.0	205.8
	15.4	12.3	12.7	12.7	14.9	20.8	11.9
	24	27	27	27	27	27	27
100	187.6	199.5	203.4	209.0	222.3	259.9	209.8
	13.7	12.5	12.4	13.4	14.6	20.0	13.7
	20	26	26	26	26	26	26
150	178.4	194.1	197.3	203.5	216.0*	253.2*	203.7
	12.8	9.5	9.9	8.8	8.5	11.9	11.4
	19	25	25	25	25	25	25

a = Mean b = Standard Deviation c = Number of rats
* = Statistically different from control mean by Dunnett's Test, Alpha = 0.05.
† = Terminal body weight minus gravid uterine weight.
These data are reproduced from Table 3, page 20 of the report.

Table 3

MEAN BODY WEIGHT GAINS (GRAMS) OF PREGNANT RATS ADMINISTERED TCP

Mg/kg/day	Days of Gestation 0-6	6-9	9-12	12-16	16-21	6-16	0-21
0	13.2[a]	5.6	8.1	15.4	41.2	29.1	83.2
	4.5[b]	2.5	3.1	3.8	6.5	5.3	10.8
	23[c]	30	30	30	30	30	23
50	14.4	5.5	7.1	13.0	39.3	25.5	78.6
	4.3	2.3	2.4	5.1	10.4	5.9	16.8
	24	27	27	27	27	27	24
100	12.5	3.9*	5.7*	13.2	37.7	22.8*	73.3
	3.6	2.0	2.8	4.8	9.6	6.4	14.8
	20	26	26	26	26	26	20
150	14.4	3.3*	6.1*	12.5*	37.2	21.9*	74.4
	4.0	3.1	2.7	3.0	8.1	5.0	14.5
	19	25	25	25	25	25	19

a = Mean b = Standard Deviation c = Number of rats
* = Statistically different from control mean by Dunnett's Test, Alpha = 0.05.
These data are reproduced from Table 4, page 21 of the report.

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There was a statistically significant increase in mean relative (gram/100 gram body weight) liver weight in high-dose (150 mg/kg/day) animals. This value was less than one standard deviation from the mean, and is probably a reflection of the decrease in body weight gain exhibited by the high-dose group. (See Table 4).

Table 4

MEAN ORGAN AND ORGAN/BODY WEIGHTS (GRAMS) OF PREGNANT RATS ADMINISTERED TCP

Dose Mg/kg/day	Final Body Wt. (G)	Liver (G)	Liver (G/100G)	Kidneys (G)	Kidneys (G/100G)
0	265.6[a]	9.71	3.66	1.40	0.53
	14.2[b]	0.66	0.15	0.16	0.05
	30[c]	30	30	30	30
50	258.0	9.52	3.69	1.38	0.53
	20.8	0.75	0.17	0.13	0.05
	27	27	27	27	27
100	259.9	9.65	3.72	1.41	0.55
	20.0	0.90	0.25	0.17	0.07
	26	26	26	26	26
150	253.2*	9.58	3.79*	1.40	0.55
	11.9	0.58	0.22	0.21	0.08
	25	25	25	25	25

a = Mean b = Standard Deviation c = Number of rats
* = Statistically different from control mean by Dunnett's Test, Alpha = 0.05.
These data are reproduced from Table 7, page 24 of the report.

Table 5 presents a summary of the reproductive parameters measured. There were no significant differences between treated and control groups. [This table is a photocopy of Table 8, page 25 of the report.]

Fetal alterations observed are presented in Table 6. [All parameters considered by the authors to be "malformations" as well as the one statistically significant alteration are included.] One control fetus had fused ribs and was missing one-half of a vertebra. One 50 mg/kg/day fetus had micrognathia. In the 100 mg/kg/day group one fetus had anophthalmia and two others from a different litter were observed to have dilated cerebral ventricles. In the 150 mg/kg/day group, one fetus had the following: microphthalmia, agnathia, maxillary aplasia and misshapen skull bones. The only statistically significant increased alteration was the incidence of vertebral spurs in the low-dose (50 mg/kg/day) group. [A table presenting a summary of historical control incidence of fetal alterations in rats was included in the report. The following are historical values for "rib-spur(s)":]

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Table 5

3,5,6-TRICHLORO-2-PYRIDINOL: ORAL TERATOLOGY STUDY IN FISCHER 344 RATS

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF BRED RATS

	3,5,6-Trichloro-2-pyridinol, mg/kg/day			
	0	50	100	150
Number of females bred	34	34	32	33
Percent pregnant[a,b]	88(30/34)	79(27/34)	81(26/32)	79(26/33)
Number of deaths	0	0	0	0
Number of litters aborted	0	0	0	0
Number of litters delivered early	0	0	0	0
Pregnancies detected by stain[c]	0	0	0	0
Number of litters totally resorbed	0	0	0	0
Litters with full term fetuses	30	27	26	25
Number of corpora lutea/dam[d]	11.1±1.8	10.9±2.1	11.0±1.5	10.7±1.7
Number of implantations/dam[d]	9.7±2.1	9.0±3.4	8.7±2.7	8.4±2.6
Percent pre-implantation loss[e]	13.6±11.8	20.3±25.5	18.3±15.5	20.6±15.0
Number of resorptions/litter[d,f]	0.2±0.5	0.3±0.5	0.2±0.5	0.2±0.4
Percent implantations resorbed	2.1(6/290)	3.3(8/244)	2.2(5/225)	2.4(5/211)
Percent litters with resorptions	16.7(5/30)	25.9(7/27)	15.4(4/26)	20.0(5/25)
Resorptions/Litters with resorptions[f]	1.2(6/5)	1.1(8/7)	1.3(5/4)	1.0(5/5)
Litter size[d]	9.5±2.0	8.7±3.3	8.5±2.7	8.2±2.7
Number of dead fetuses	0	0	0	0
Sex Ratio (M:F, %)	46:54	43:57	51:49	55:45
Fetal body weights (grams)[g]	4.46±0.11	4.49±0.37	4.43±0.20	4.45±0.13
Gravid uterine weight (grams)[d]	57.18±10.84	52.25±18.38	50.12±14.61	49.48±14.52

[a] Number of females with implantations/total number bred.

[b] Includes two controls and one in the 150 mg/kg/day dose group which were submitted for necropsy on Day 6 of gestation and excluded from data analyses.

[c] No. of females detected as being pregnant only after staining the uterus with sodium sulfide stain/total no. stained.

[d] Mean ± S.D.

[e] Mean percent per litter ± S.D.

[f] Not statistically analyzed.

[g] Mean of litter means ± S.D.

No values were statistically different from the controls, α=0.05.

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Table 6

INCIDENCE OF FETAL ALTERATIONS AMONG LITTERS OF RATS ADMINISTERED TCP

mg/kg/day	0	50	100	150
External Examination	284 (30)	236 (27)	220 (26)	206 (25)
Soft Tissue Examination	147 (30)	125 (27)	119 (26)	113 (25)
Skeletal Examination	284 (30)	236 (27)	220 (26)	205 (25)[a]
Bones of the Skull[b]	137 (30)	111 (24)	101 (24)	93 (24)
External Observations				
Anophthalmia+	0[F]	0	0.5 (1)	0
	0[L]	0	3.8 (1)	0
Microphthalmia+, Agnathia+	0	0	0	0.5 (1)[c]
Maxillary Aplasia+	0	0	0	4.0 (1)
Micrognathia+	0	0.4 (1)	0	0
	0	3.7 (1)	0	0
Visceral Observations				
Dilated cerebral ventricles+	0	0	1.7 (2)	0
	0	0	3.8 (1)	0
Skeletal Observations				
Skull, misshapen+	0	0	0	1.1 (1)[c]
	0	0	0	4.2 (1)
Vertebrae, missing 1/2+	0.4 (1)[d]	0	0	0
	3.3 (1)	0	0	0
Ribs, extra-cervical+	0	0	0	0.5 (1)
	0	0	0	4.0 (1)
Ribs, fused+	0.4 (1)[d]	0	0	0
	3.3 (1)	0	0	0
Ribs, spur(s)	2.5 (7)	6.8 (16)*	4.1 (9)	6.3 (13)
	23.3 (7)	51.9 (14)	26.9 (7)	28.0 (7)

a=One fetus at 150 mg/kg/day disarticulated, could not be evaluated.
b=All fetuses from litters with less than 4 pups examined viscerally & heads removed & fixed in Bouin's solution.
c-d=Alterations with same superscript were observed in the same fetus.
+=Considered to be a malformation. F=Fetuses L=Litters
*=Significantly different from control value, alpha = 0.05.
These data were extracted from Table 9, pages 26-28 of the report.

HISTORICAL VALUES FOR "RIB-SPURS"

	Incidence	Range
Fetuses	115/3656	0/287 - 19/164
Litters	83/401	0/31 - 17/27

These data were extracted from Table 10, page 29 of the report.

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IV. Discussion

The only apparent treatment related effect on pregnant rats was a decrease in body weight gain during the time of dosing (days 6 through 15 of gestation) in mid- (100 mg/kg/day) and high- (150 mg/kg/day) dose groups. A review of individual body weight gains during the dosing period indicates that there were a larger number of controls which gained more grams than the 100 or 150 mg/kg/day females: gain of at least 30 grams - 13/30 controls, 4/26 mid dose, 2/25 high dose; at least 25 grams - 21/30 controls, 11/26 mid dose, 6/25 high dose; below 20 grams - 1/30 controls, 7/26 mid dose, 6/25 high dose. The mean corrected body weight gain ([final body weight minus gravid uterus weight] - initial body weight) was: control, 27.6 gm; 50 mg/kg, 27.3 gm; 100 mg/kg, 22.2 gm; 150 mg/kg, 25.3 gm. There appears to be less of a mean gain in the mid-dose group, but as there does not seem to be a dose response, this difference is considered to be a biological variation. Although there was a statistically significant increase in relative (gram/100 grams body weight) liver weight at 150 mg/kg/day, it is felt that this was a reflection of the decrease in body weight gain.

It is considered that the "malformations" described are of such a low incidence (both regarding the number of fetuses as well as the number of litters involved) that they are not considered to be related to administration of TCP. One fetus in the 150 mg/kg/day group was severely malformed (microphthalmia, agnathia, maxillary aplasia and misshapen skull bones). Although historical data from the laboratory indicated microphthalmia to have been observed, there were no fetuses with agnathia or misshapen skull bones (no mention of maxillary aplasia in historical data). It is this reviewer's opinion that the severely malformed fetus was most likely not caused by dosing with TCP.

There was no observable difference between treated and control groups regarding any of the parameters examined at Cesarean section. The statistically increased number of fetuses with "rib-spur(s)" in rats administered 50 mg/kg/day (low dose), was the only apparent alteration in offspring. Because the number is relatively low, within historical range and is significant only in the low-dose group, it is most likely that this observation falls within normal biological variation.

V. Recommendation

The Maternal Toxicity No Observed Effect Level (NOEL) is 50 mg/kg/day. The Maternal Toxicity Lowest Observed Effect Level (LOEL) is 100 mg/kg/day (mid dose). There appeared to be a group mean decrease in bodyweight gain during dosing (days 6 through 15 of gestation) in the mid- (100 mg/kg/day) and high - (150 mg/kg/day) dose groups. There were no apparent dosing related effects regarding any other parameters.

The Developmental Toxicity No Observed Effect Level (NOEL) is 150 mg/kg/day (the highest dose tested - HDT). The Developmental Toxicity Lowest Observed Effect Level (LOEL) is >150 mg/kg/day. There were no apparent compound related differences regarding any Developmental Toxicity parameters in any dose group.

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The study is acceptable and is considered to be Core Minimum.

No treatment-related effects on any of the reproductive parameters measured were observed at any dose level (Scortichini et al., 1986).

The purpose of the study described herein was to evaluate the embryotoxic and teratogenic potential of trichloropyridinol administered orally to rats. This study was conducted in accordance with the spirit of the Good Laboratory Practice Procedures for Non-Clinical Studies (FDA, 1978), the Environmental Protection Agency (EPA): FIFRA Good Laboratory Practice Procedures (EPA, 1983), the Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals (NTIS, 1982), and the Standard Operating Procedures of The Dow Chemical Company Mammalian and Environmental Toxicology Research Laboratory.

MATERIALS AND METHODS

Test Material. The test material was obtained from the Agricultural Products Department, The Dow Chemical Company, Midland, MI and identified as AGR #143197. The sample was assayed to be 99.7% pure by differential scanning calorimetry (DSC) (Hummel, 1985; Pasztor, 1987). The oral route of administration was chosen since it is a likely route of potential human exposure. Trichloropyridinol and its sodium salt have limited solubility in water and corn oil, making it difficult to prepare solutions of either compound in these vehicles. In order to quantitatively administer the compound by gavage, suspension of trichloropyridinol in 0.5% aqueous METHOCEL was selected as the method of administration. Analysis of a suspension of 25 mg trichloropyridinol/ml of METHOCEL showed the suspension to be homogeneous throughout and stable for at least 76 days (Kastl, 1985).

Test Animals. Stock supplies of adult male and female Fischer 344 rats were obtained from Charles River Breeding Laboratories (Kingston, NY). The Fischer 344 rat was chosen as a test species based on its general acceptance for developmental toxicity testing, reliable commercial supplier, and availability of historical data. Upon receipt at the

laboratory[1], all animals were examined for health status by the laboratory veterinarian and acclimated to the laboratory conditions. The animal rooms of the testing facility were designed to maintain adequate environmental conditions concerning temperature, relative humidity, airflow and lighting and were regulated for the species on test. Adult virgin females (approximately 150-225 g) were bred overnight with males of the same strain (one male to one female) with Day 0 of gestation determined by the presence of sperm in vaginal smears. Randomization of test animals, grouped according to their Day 0 of gestation, was performed using computer-generated tables of random numbers. Animals were uniquely identified by means of numbered metal ear tags. All animals were allowed access to Certified Laboratory Rodent Chow No. 5002 (Ralston Purina Company, St. Louis, MO) and tap water ad libitum. Water and feed analyses were done according to the Standard Operating Procedures of the Mammalian and Environmental Toxicology Research Laboratory.

Compound Preparation and Administration. Trichloropyridinol was administered as a suspension in 0.5% aqueous METHOCEL. Test suspensions were prepared such that a dose volume of 4 ml/kg yielded the appropriate dose. Dosing suspensions were prepared once during the course of the study based on the stability data. A control group was administered the 0.5% METHOCEL vehicle. The test suspension for each dose level was analyzed to determine the concentration of the test material once during the conduct of the study.

Experimental Design. Groups of 32-34 bred rats were administered trichloropyridinol by oral gavage on Days 6 through 15 of gestation at dose levels of 50, 100, or 150 mg/kg/day. A control group of 34 bred rats was given the 0.5% METHOCEL vehicle during the same period. These dose levels were selected based upon the results of the probe study discussed previously (Scortichini et al., 1986). Body weights were recorded on Day 0, daily on Days 6 through 16, and on Day 21 of gestation. Dose volumes during the treatment period were adjusted daily.

[1]Fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

All animals were observed daily for indications of treatment-related effects due to the test material. Statistical analyses of body weights and weight gains were conducted using data recorded on Days 0, 6, 9, 12, 16 and 21 of gestation. Cesarean section of bred rats was performed on Day 21 of gestation, and maternal liver, kidney and gravid uterine weights were recorded at the time of cesarean section. Sections of maternal liver and kidneys were preserved in neutral, phosphate-buffered 10% formalin, but histopathologic examination was not performed.

Fetal Observation. Test animals were sacrificed by carbon dioxide inhalation on Day 21 of gestation, the uterine horns were exteriorized through an abdominal incision and the following data recorded: 1) number and position of fetuses in utero, 2) number of live and dead fetuses, 3) number and position of resorption sites, 4) the number of corpora lutea, 5) the sex and body weight of each fetus, and 6) any gross external alteration. The uteri of apparently non-pregnant animals were stained with a 10% solution of sodium sulfide (Kopf et al., 1964) and examined for evidence of implantation sites. One-half of each litter, selected using a table of random numbers, was examined by dissection under a low power stereomicroscope for evidence of soft tissue alterations (Staples, 1974). The heads of rat fetuses examined by dissection were removed, placed in Bouin's fixative and examined by the serial sectioning technique of Wilson (1965). All fetuses were then preserved in alcohol, eviscerated and subsequently cleared and stained with alizarin red-S (Dawson, 1926) and examined for skeletal alterations.

Statistical Evaluation. Maternal and fetal body weights, maternal weight gains, and absolute and relative organ weights were evaluated by Bartlett's test for equality of variance. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant, analysis by Dunnett's test or the Wilcoxon Rank Sum test with Bonferroni's correction was performed. Statistical evaluation of the frequency of pre-implantation loss, resorptions and alterations among litters and the fetal population was performed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implantations, and litter size were analyzed using the

Wilcoxon Rank-Sum test. The pregnancy rate was analyzed by the Fisher exact probability test. The fetal sex ratio was analyzed by a binominal distribution test. Statistical outliers were identified by a sequential outliers test; however, except for feed and water consumption, outliers were not excluded from analyses unless justified by documented, scientifically sound reasons unrelated to treatment.

The nominal alpha levels used were as follows:

Bartlett's Test of Variance (Winer, 1971)	α=0.01
Analysis of Variance (Steel and Torrie, 1960)	α=0.10
Nonparametric ANOVA (Hollander and Wolfe, 1973)	α=0.10
Dunnett's Test (Winer, 1971)	α=0.05, two-sided
Wilcoxon Rank-Sum Test (Hollander and Wolfe,1973)	α=0.05, two-sided with Bonferroni correction (Miller, 1966)
Fisher's Test (Siegel, 1956)	α=0.05, one-sided
Censored Wilcoxon Test (Haseman and Hoel, 1974)	α=0.05, one-sided
Outlier Test (Grubbs, 1969)	α=0.02, two-sided

Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was much greater than the cited alpha levels would suggest. Therefore, the final interpretation of the numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in light of other biologic and pathologic findings.

RESULTS

A summary of the analyses of the dosing suspensions used in this study is presented in Table 1. Analysis using liquid chromatography revealed that each suspension used was within 103 to 108 % of the targeted concentration.

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Primary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy 4/27/88
Review Section V/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui 4/29/88
Acting Section Head, Review Section V

I. Study Type: Teratogenicity Study (Developmental Toxicity)
(Guideline § 83-3)

Study Title: 3,5,6-Trichloro-2-Pyridinol: Oral Teratology Study in New Zealand White Rabbits

EPA Identification:

EPA Identification: 3H5396
EPA Accession: 403488-03
EPA Record: 205144
Caswell: 821AA
Tox. Branch Project: 8-0213

Sponsor: The Dow Chemical Company
Midland, MI 48674

Testing Laboratory: Mammalian and Environmental Toxicology
Research Laboratory
Health and Environmental Sciences
The Dow Chemical Company
Midland, MI 48674

Study Number: K-038278-015

Study Date: July 23, 1987

Study Authors: T. R. Hanley, M.S., D.A.B.T., G. J. Zielke, B.S., and L. G. Lomax, D.V.M., Ph.D., D.A.C.V.P.

Recommendation: The Maternal Toxicity No Observed Effect Level (NOEL) is 100 mg/kg/day. The Maternal Toxicity Lowest Observed Effect Level (LOEL) is 250 mg/kg/day (highest dose tested - HDT). There was an apparent mean body weight loss in the 250 mg/kg/day group only during the period of dosing (days 7 through 19 of gestation). There was no apparent compound effect on corrected body weight (body weight gain - gravid uterus weight) when measured from day 0 until day 28 (Cesarean Section). There were no suggestive group differences regarding any other parameters.

The Developmental Toxicity NOEL is 25 mg/kg/day. The Developmental Toxicity LOEL is 100 mg/kg/day. The only apparent Developmental Toxicity parameter which was suggestive of compound administration was an increase in the number of fetuses and litters in both the mid- (100 mg/kg/day) and high- (250 mg/kg/day) dose groups which were reported to have hydrocephaly or hydrocephaly/dilated cerebral ventricles when compared with this study's control group or the laboratory's historical data.

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Core Classification: Supplemental [This may be upgraded to Core Minimum provided that answers to the artificial insemination questions are acceptable.]

Maternal Toxicity NOEL = 100 mg/kg/day
Maternal Toxicity LOEL = 250 mg/kg/day
Developmental Toxicity NOEL = 25 mg/kg/day
Developmental Toxicity LOEL = 100 mg/kg/day

Test Material:

Name: 3,5,6-trichloro-2-pyridinol (TCP)

Lot No.: AGR # 143197

Purity: 99.7%

Vehicle: 0.5% aqueous METHOCEL A4M

NOTE: The material is the major degredation product of chlorpyrifos, the active ingredient in DURSBAN and LORSBAN brand insecticides.

II. Materials and Methods

The test material was suspended in a vehicle of 0.5% aqueous METHOCEL and administered to rabbits by oral gavage at a volume of 2 ml per kg body weight. The control group received the 0.5% METHOCEL vehicle. Homogeneity and stability were tested with 25 mg/ml of METHOCEL and were found to be satisfactory for 76 days (Kastl, P. E., 1985, Analytical report No. HET K-38278-6, The Dow Chemical Company). Dose suspensions were prepared once and analyzed once during the study.

Adult male and female New Zealand White rabbits were obtained from Hazleton-Dutchland, Inc., Denver, PA. Animals were acclimated to laboratory conditions for a minimum of three weeks prior to insemination. The rabbits were housed individually; were given 4 oz of food per day prior to the start of the study; had food increased to 8 oz per day during the study for increased nutritional demands during pregnancy; and had tap water available ad libitum.

All females (3.5-4.5 kg) were injected I.V. (marginal ear vein), three weeks prior to the date of insemination, with 50 I.U. (0.1 ml) of chorionic gonadotropin (HCG) in order to synchronize estrous. The females were artificially inseminated with the day of insemination being Day 0 of gestation. A second administration of 50 I.U. of HCG was given on Day 0 to induce ovulation. Computer-generated randomization, according to insemination date, was used to group animals.

There were no details provided concerning the insemination procedure. How many males were used? Which females received sperm from which males? Did the reported fetal anomalies come from mothers which were inseminated by the same male (could the anomalies have been male-mediated rather than female-mediated)?

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Sixteen inseminated rabbits were placed in each group and administrated TCP on Days 7 through 19 of gestation at dose levels of 0, 25, 100 or 250 mg/kg/day. [The dose levels were based upon results of a prior probe study.] Body weights were recorded on Day 0, daily on Days 7 through 20, and on Day 28 of gestation. (Statistical analyses of body weights and weight gains were performed utilizing data from Days 0, 7, 10, 13, 16, 20 and 28). Dose volumes were adjusted daily. Rabbits were observed daily for possible treatment-related effects. Cesarean sections were performed on Day 28 of gestation with maternal liver, kidney and gravid uterine weights recorded. Sections of livers and kidneys were preserved in phosphate-buffered 10% formalin. Rabbits which died, had pregnancy terminated early or were found moribund were submitted for gross pathologic examination.

At Cesarean section on day 28 of gestation, the following data were recorded: number and position of fetuses in utero; number of live and dead fetuses; number and position of resorption sites; number of corpora lutea; sex and body weight of each fetus; and any gross external alterations. Uteri of apparently non-pregnant rabbits were stained with a 10% solution of sodium sulfide and examined for evidence of implantation sites. All fetuses were examined by dissection under a low power stereomicroscope for evidence of soft tissue alterations. All fetuses were then preserved in alcohol, eviscerated, cleared, stained with alizarin red-S and examined for skeletal alterations.

Detailed descriptions of statistical analyses employed were described.

A Quality Assurance statement was included.

A copy of the Materials and Methods section from the report is appended.

There are no additional comments regarding the Materials and Methods section. (See questions regarding insemination.)

III. Results

A. Analysis of Dosing Suspensions

Table 1 presents analyses of the dosing suspensions. Liquid chromatography indicated that suspensions used were 100-105% of the desired concentrations.

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Table 1

SUMMARY OF 3,5,6-TRICHLORO-2-PYRIDINOL DOSE SOLUTION ANALYSES[a]

Targeted Dose (mg/kg/day)	% of Targeted Concentration Mean ± S.D.
Control	ND[b]
25	102±4
100	100±4
250	105±5

a = Mean of 2 injections of 1 aliquot per sample.
b = N.D. - not detected, detection limit 0.7 mg/g Methocel suspension.
This table is reproduced from Table 1, page 18 of the report.

B. Clinical Observations

There were no clinical signs which appeared to be attributable to compound administration. One low-dose (25 mg/kg/day) rabbit (No. 86A-9739) aborted on Day 25 and was sent to necropsy. Also, one high-dose (250 mg/kg/day) rabbit (No. 86A-9778) aborted on day 23 and was sent to necropsy.

C. Body Weight

Tables 2 and 3 are photocopied from Tables 3 and 4, pages 20 and 21 of the report, and present mean group body weights and body weight gains for times or time periods throughout the study. There was a statistically significant mean difference for weight gains during dosing (Days 7 through 19) between the control (+139.4±185.0 S.D.) and the high-dose (250 mg/kg/day) animals (-66.8±194.1). Attention should be drawn to the relatively large S.D. in relation to the mean values. In all four groups, there were individual rabbits which lost weight as well as those which gained.

Table 4 presents data concerning body weight gain/loss in the pregnant rabbits. The gravid uterine weights were similar in all four groups (the mean number of fetuses and fetal body weights were also about the same). In each group some animals gained weight, but the majority lost. The body weight gains/losses (minus the gravid uteri) ranged from gains of 233-425 grams to losses of 464-797 grams. Taking these factors into consideration, it appears that there was little or no difference in corrected body weight gain between any of the four groups and that the majority of the rabbits lost some weight from day 0 to day 28 (the duration of the study). Therefore, it appears that the administration of TCP at the doses tested caused an effect on maternal body weight only during the dosing period.

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Table 2

3,5,6-TRICHLORO-2-PYRIDINOL: ORAL TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

MEAN BODY WEIGHTS (G) OF PREGNANT RABBITS

DOSE MG/KG/DAY		DAY OF GESTATION 0	7	10	13	16	20	28	28(C)
0	MEAN	3794.7	3937.3	3963.8	4035.0	4076.6	4076.7	4126.8	3695.7
	S.D.	196.8	251.7	203.0	214.3	222.8	238.7	314.3	292.9
	N=	15	15	15	15	15	15	15	15
25	MEAN	3812.9	4059.3	4049.9	4103.7	4164.1	4144.0	4179.5	3749.7
	S.D.	180.1	283.0	254.4	301.8	336.1	344.1	380.7	419.9
	N=	16	16	16	16	16	16	15	15
100	MEAN	3819.2	4012.7	4002.2	4046.1	4074.1	4078.3	4146.0	3700.5
	S.D.	187.7	237.4	215.7	243.9	193.7	257.3	147.8	178.3
	N=	13	13	13	13	13	13	13	13
250	MEAN	3813.8	4020.6	3980.8	4014.1	4020.2	3953.9	4102.3	3681.0
	S.D.	191.7	192.6	175.0	197.4	198.2	217.3	211.2	200.2
	N=	14	14	14	14	14	14	13	13

THERE WERE NO STATISTICALLY IDENTIFIED DIFFERENCES FROM CONTROL MEAN.
28(C) = TERMINAL BODY WT. - GRAVID UTERINE WT.

Table 3

3,5,6-TRICHLORO-2-PYRIDINOL: ORAL TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

MEAN BODY WEIGHT GAINS (G) OF PREGNANT RABBITS

DOSE MG/KG/DAY		DAYS OF GESTATION 0-7	7-10	10-13	13-16	16-20	20-28	7-20	0-28
0	MEAN	142.6	26.6	71.2	41.6	0.1	50.1	139.4	332.1
	S.D.	160.9	92.5	52.1	51.5	92.5	139.8	185.0	266.7
	N=	15	15	15	15	15	15	15	15
25	MEAN	246.4	-9.4	53.8	60.3	-20.1	-0.4	84.6	368.1
	S.D.	130.9	73.3	101.8	74.6	89.7	148.5	175.7	244.0
	N=	16	16	16	16	16	15	16	15
100	MEAN	193.5	-10.5	43.9	28.0	4.2	67.6	65.7	326.8
	S.D.	125.1	67.3	81.7	70.1	122.9	149.5	161.3	154.2
	N=	13	13	13	13	13	13	13	13
250	MEAN	206.8	-39.8	33.3	6.1	-66.3	124.8	-66.8*	267.1
	S.D.	95.2	54.4	77.2	99.6	86.2	134.2	194.1	222.2
	N=	14	14	14	14	14	13	14	13

* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA = 0.05.

Table 4

BODY WEIGHT GAINS (LOSSES) OF PREGNANT RABBITS RECEIVING TCP

Dose mg/kg	Mean Initial B.W.	Mean Day 28 Final B.W. (with Gr. Uterus)	Mean Gravid Uterine Weight	Mean B.W. Gain	Mean B.W. Gain Corrected †	No. Lost Wt./Total No. with Full Term Fetuses
0	3794.7	4126.8	431.08	332.1	- 99	10/15
25	3812.9	4179.5	429.86	368.1	- 63	11/15
100	3819.2	4146.0	445.45	326.8	- 119	10/13
250	3813.8	4102.3	421.29	267.1	- 133[a]	9/13

NOTE: All body and uterine weights are in grams.
† = Corrected B.W. gain = B.W. gain - uterine weight
a = This value is the result of: 4102.3 - 3813.8 = 288.5; 421.29 - 288.50 = 132.79.

These data are extracted and/or calculated from data presented in Tables 3 (page 20), 6 (page 23) and A2 (pages 36 - 40) of the report.

D. Organ Weights

Absolute (grams) and relative (grams/100 grams body weight) liver and kidney weights are presented in Table 5. [This table is a photocopy of Table 5, page 22 of the report.] There did not appear to be any compound related effects on these organ weights.

E. Reproductive Parameters

A summary of the reproductive parameters measured is presented in Table 6. [This table is a photocopy of Table 6, page 23 of the report.] There were no apparent differences between treated and control groups regarding preimplantation loss, postimplantation loss, fetal body weights, sex ratio and litter size.

F. Fetal Anomalies

Table 7 presents the fetal anomalies considered to be "malformations" by the study authors and Table 8 presents the fetal anomalies considered to be "variations" by the authors (alterations listed are those observed to occur in two or more fetuses in a group). [Refer to the "Federal Register, September 24, 1986, Volume 51, No. 185, pages 34028-34040; Part V, Environmental Protection Agency, Guidelines for the Health Assessment of Suspect Developmental Toxicants", for developmental end points, variations and incidence of observations.] There appears to be the possible indication of central nervous system

Table 5

3,5,6-TRICHLORO-2-PYRIDINOL: ORAL TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

MEAN ORGAN AND ORGAN BODY WEIGHTS (G) OF PREGNANT ANIMALS

DOSE MG/KG/DAY		FINAL BODY WT.(G)	LIVER (G)	LIVER (G/100G)	KIDNEYS (G)	KIDNEYS (G/100G)
0	MEAN	4126.8	100.18	2.44	19.40	0.47
	S.D.	314.3	11.05	0.30	2.65	0.07
	N=	15	15	15	15	15
25	MEAN	4179.5	107.36	2.56	18.91	0.45
	S.D.	380.7	24.98	0.48	2.36	0.04
	N=	15	15	15	15	15
100	MEAN	4146.0	98.28	2.37	18.81	0.45
	S.D.	147.8	17.37	0.42	2.21	0.06
	N=	13	13	13	13	13
250	MEAN	4102.3	106.19	2.61	19.54	0.48
	S.D.	211.2	20.59	0.62	2.08	0.06
	N=	13	13	13	13	13

THERE WERE NO STATISTICALLY IDENTIFIED DIFFERENCES FROM CONTROL MEAN.

Table 6

3,5,6-TRICHLORO-2-PYRIDINOL: ORAL TERATOLOGY STUDY IN NEW ZEALAND WHITE RABBITS

Observations Made at the Time of Cesarean Section of Inseminated Rabbits

	3,5,6-Trichloro-2-pyridinol, mg/kg/day			
	0	25	100	250
Number of females bred	16	16	16	16
Percent pregnant[a]	94(15/16)	100(16/16)	88(14/16)	88(14/16)
Number of deaths	0	0	0	0
Number of litters aborted	0	1	0	1
Number of litters delivered early	0	0	0	0
Pregnancies detected by stain[b]	0/1	0	0/2	0/2
Number of litters totally resorbed	0	0	1	0
Litters with full term fetuses	15	15	13	13
Number of corpora lutea/dam[c]	10.4±1.5	11.5±2.7	11.8±3.0	11.0±2.3
Number of implantations/dam[c]	8.7±2.0	8.5±3.1	8.7±3.2	8.6±1.9
Percent pre-implantation loss[d]	17.1±15.5	25.5±24.5	23.4±22.3	20.9±15.9
Number of resorptions/litter[c,e]	0.9±1.2	0.5±0.7	1.1±1.4	1.0±1.4
Percent implantations resorbed	10.0(13/130)	5.5(7/127)	12.3(15/122)	11.6(13/112)
Percent litters with resorptions	46.7(7/15)	33.3(5/15)	57.1(8/14)	53.8(7/13)
Resorptions/litters with resorptions[e]	1.9(13/7)	1.4(7/5)	1.9(15/8)	1.9(13/7)
Litter size[c]	7.8±2.2	8.0±3.3	7.6±3.7	7.6±2.4
Number of dead fetuses	0	0	0	1
Sex Ratio (M:F, %)	50:50	43:57	42:58	47:53
Fetal body weights (grams)[f]	35.46±5.88	36.00±5.90	36.20±6.60	34.38±6.38
Gravid uterine weight (grams)[c]	431.08±95.42	429.86±143.67	445.45±123.35	421.29±123.90

[a] Number of females with implantations/total number bred.
[b] Number of females detected as being pregnant only after staining the uterus with sodium sulfide stain/total number stained.
[c] Mean ± S.D.
[d] Mean percent per litter ± S.D.
[e] Not statistically analyzed.
[f] Mean of litter means ± S.D.
No values differed significantly from the control value.

Table 7

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ANOMALIES REPORTED IN FETUSES FROM RABBITS ADMINISTERED TCP DURING DAYS 7-19 OF GESTATION

Group (Mg/kg/day)	Mother (Number)	Implant (Number)	Anomalies
0	86A-9718	2	Dilated renal pelvis-right
	86A-9723	1	Ribs-calloused #7,8-bilateral
	86A-9726	6	Dilated cerebral ventricles
25	N O N E	R E P O R T E D	
100	86A-9747	4	Hydrocephaly
	86A-9759	7	Fused aorta and pulmonary artery, stenosis of pulmonary artery, distension of aortic arch, ventricular septal defect
	86A-9760	1	Micrognathia, cleft palate, hydrocephaly, scoliosis
		3	Dilated cerebral ventricles, scoliosis
		4	Hydrocephaly
	86A-9761	8	Rib-calloused #6-left
	86A-9762	9	Pulmonary hypoplasia-all lobes, retroesophageal right subclavian artery, absence of inominate artery, agenesis right kidney, thoracic ascites, left kidney in pelvic region
250	86A-9763	1	Hydrocephaly
		2	Dilated cerebral ventricles
	86A-9765	3	Ribs-calloused #7,8-right
	86A-9768	6	Fused lungs, missing azygomatic lobe of lung
	86A-9769	6	Dilated cerebral ventricles
	86A-9777	10	Hydrocephaly
DEAD FETUS			
250	86A-9775	5	DEAD FETUS - Cleft palate, severe forelimb flexure, hydrocephaly, craniochisis, scoliosis

[These findings were extracted from the "Results" section of the text of the report as well as the APPENDIX (Individual Animal Data - report pages 49-112.)]

Table 8

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FETAL ALTERATIONS (NOT CONSIDERED MALFORMATIONS) OBSERVED AMONG LITTERS OF RABBITS ADMINISTERED TCP
(Observations Listed Only When Alterations Occurred in Two or More Fetuses in a Group)

		TCP (mg/kg/day)				Historical Data*		
		0	25	100	250	Incidence	%	Total Examined
Number Fetuses (Litters) Examined		117(15)	120(15)	107(13)	99(13)			
OBSERVATION								
Forelimb Flexure - Mild	F	0.9(1)†	0	1.9(2)	0	6	0.09	6497[a]
	L	6.7(1)	0	7.7(1)	0	6	0.71	840[b]
Lung(s)-Missing Azygomatic Lobe	F	0	0	0	3.0(3)	1	0.03	2936
	L	0	0	0	15(2)	1	0.12	839
Skull-Irregular Pattern of Ossif.	F	2.6(3)	0.8(1)	0	2.0(2)	6	0.09	6456
	L	20(3)	6.7(1)	0	15(2)	3	0.36	836
Skull-Hyoid-Delayed Ossification	F	48(56)	53(64)	53(57)	49(48)	418	6.47	6456
	L	87(13)	100(15)	100(13)	92(12)	128	15.31	836
Skull-Hyoid-Crooked	F	0.9(1)	0	0.9(1)	3.0(3)	53	0.82	6456
	L	6.7(1)	0	7.7(1)	23(3)	38	4.55	836
Vertebrae-Delayed Ossification Dentoid Process	F	0.9(1)	3.3(4)	5.6(6)	1.0(1)	5	0.08	6456
	L	6.7(1)	13(2)	31(4)	7.7(1)	5	0.60	836
Vertebrae-Extra Site of Ossification	F	0	1.7(2)	0	0	7	0.11	6456
	L	0	13(2)	0	0	7	0.84	836
Centra-Delayed Ossification	F	0	0	0	2.0(2)	19	0.29	6456
	L	0	0	0	7.7(1)	18	2.15	836
Ribs-Spur(s)	F	27(32)	20(24)	20(21)	15(15)	102[c]	1.58	6456
	L	87(13)	67(10)	77(10)	54(7)	56	6.70	836
Sternebrae-Delayed Ossification	F	35(41)	31(37)	50(53)	32(32)	3263	50.54	6456
	L	80(12)	60(9)	85(11)	85(11)	722	86.36	836
Sternebrae-Fused	F	0	0	0	2.0(2)	40	6.20	6456
	L	0	0	0	7.7(1)	36	4.31	836

F = Fetuses L = Litters
* = Data from 46 studies.
† = Percent (number)
a = Number of fetuses examined. b = Number of litters examined.
c = Lumbar - other 309/184
Data extracted from Table 7, pages 24-29, and Historical Control Data pages 113-148 of the report.

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(CNS) involvement with four fetuses from two litters (three in one litter) in the 100 mg/kg/day group and four fetuses from three litters (plus one dead fetus from a fourth litter with cleft palate, severe forelimb flexure, hydrocephaly, chranioschisis and scoliosis) in the 250 mg/kg/day group with hydrocephaly or dilated cerebral ventricles versus one fetus in the control group (dilated cerebral ventricles).

Fetal alterations which were observed in greater numbers in dose groups versus control (Table 8) were as follows: 25 mg/kg/day (low dose) = vertebrae delayed ossification of dentoid process, 4 fetuses in 2 litters vs 1 in 1 control; 100 mg/kg/day (mid dose) = vertebrae delayed ossification of dentoid process, 6 fetuses in 4 litters vs 1 in 1 control; 250 mg/kg/day (high dose) = lung(s) missing azygomatic lobe, 3 fetuses in 2 litters vs 0 in control - centra delayed ossification 2 fetuses in 1 litter vs 0 in control - sternebrae fused, 2 fetuses in 1 litter vs 0 in control.

IV. Discussion

Administration of 250 mg/kg/day of TCP to pregnant rabbits on Days 7 through 19 of gestation appeared to cause a mean body weight loss of about 77 grams compared with a mean increase of about 139 grams in the control group (the 25 and 100 mg/kg/day groups gained a mean of 85 and 66 grams, respectively). During the dosing period, some pregnant rabbits in all groups had weight losses and some had weight gains so that within a group they had the following ranges: control, -6 to +500 grams; 25 mg/kg/day dose, -170 to +251; 100, -324 to +263; and 250, -412 to +211. Even though fluctuations in weight gain are common in rabbits, the results observed in this study seem to indicate that 250 mg/kg/day of TCP administered on Days 7 through 19 of gestation did have an effect on body weight gain during the dosing period. However, considering corrected body weight gains (minus gravid uterus) as indicated in Table 4, TCP dosing did not appear to have an overall effect on maternal body weight. There were no significant differences observed in any of the reproductive parameters examined.

TCP administration appeared to have possibly caused fetal "malformations" involving the central nervous system (CNS) and neural tube. Hydrocephaly (H) or dilated cerebral ventricles (DCV) were observed in a larger number of fetuses and/or litters in the mid-dose (100 mg/kg/day) and high-dose (250 mg/kg/day) groups compared with the control group: control - one fetus in one litter (DCV); low dose - none; mid dose - 4 fetuses (3-H, 1-DCV) in 2 litters (1-H in 1 and 2-H plus 1-DCVin 1); and high dose - 4 fetuses (2-H plus 2-DCV = 1-H plus 1 DCV in one litter; 1-H in one litter; 1-DCV in one litter). In addition, one 250 mg/kg/day litter had a dead fetus which was observed to have cleft palate, severe forelimb flexure, hydrocephaly, chranioschisis and scoliosis.

Table 9 presents the number and percent of litters and fetuses with central nervous system anomalies. The mid- and high-dose groups had 15.4% (2/13) and 23.1% (3/13), respectively, of the litters (with full term fetuses) showing CNS anomalies vs 6.7% (1/15) in the control group. Hydrocephaly appeared in 7.7% (1/13) mid-dose and 15.4% (2/13)

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Table 9

NUMBER AND PERCENT OF RABBIT LITTERS AND FETUSES WITH CENTRAL NERVOUS SYSTEM ANOMALIES - TCP ADMINISTRATION

mg/kg/day	0	25	100	250
No. litters with full term fetuses	15	15	13	13
No. litters (%) with CNS anomalies	1(6.7)	0	2(15.4)	3(23.1)[a]
hydrocephaly (H)	0	0	1(7.7)	2(15.4)[b]
dilated cerebral ventricles (DCV)	1(6.7)	0	1(7.7)	2(15.4)[c]
No. fetuses examined	117	120	107	99
No. fetuses (%) with CNS anomalies	1(0.9)	0	4(3.7)	4(4.0)
hydrocephaly (H)	0	0	3(2.8)	2(2.0)[d]
dilated cerebral ventricles (DCV)	1(0.9)	0	1(0.9)	2(2.0)

a = Excluding one litter with one dead fetus having multiple anomalies including CNS.
b = One litter also with DCV.
c = One litter also with H.
d = Excluding one dead fetus with H.

Table 10

CENTRAL NERVOUS SYSTEM ANOMALIES IN HISTORICAL CONTROL RABBITS

		Incidence	%
Hydrocephaly	F[a]	1/2936	0.03
	L[b]	1/839	0.12
Cerebral Ventricles Dilated	F	3/2936	0.10
	L	2/839	0.24

a = Fetus b = Litter
[These data are extracted from Table 8, page 28 of the report.]

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high-dose litters vs 0% in control. Dilated cerebral ventricles appeared in 7.7% (1/13) mid-dose and 15.4% (2/13) high-dose litters vs 1 control (6.7%). Regarding fetuses, those with CNS anomalies were 3.7% (4/107) and 4.0% (4/99) in mid- and high-dose groups, respectively, vs 0.9% (1/15) in controls. The authors reported one case of hydrocephaly and three of severely dilated cerebral ventricles in about three thousand control fetuses (0.03%) from 839 litters (0.12%); Table 10. It therefore appears that the mid (100 mg/kg/day) and high (250 mg/kg/day) doses of TCP may have been responsible for the increased incidence observed.

Fetal alterations of delayed ossification in the mid-dose group as well as missing azygomatic lung lobes, delayed ossification of the vertebral centra and fused sternebrae in the high-dose group, are, by themselves, not considered by this reviewer to be definitively a result of TCP administration. However, taking into consideration the categories of developmental toxicity, it appears that the high dose of 250 mg/kg/day did have an effect on fetal development. Conservatively, it is believed that the mid dose of 100 mg/kg/day may also have had an effect on fetal development due to similar findings (CNS anomalies).

V. Recommendation

The Maternal Toxicity No Observed Effect Level (NOEL) is 100 mg/kg/day. The Maternal Toxicity Lowest Observed Effect Level (LOEL) is 250 mg/kg/day (highest dose tested - HDT). There was an apparent mean body weight loss in the 250 mg/kg/day group only during the period of dosing (days 7 through 19 of gestation). There was no apparent compound effect on corrected body weight (body weight gain - gravid uterus weight) when measured from day 0 until day 28 (Cesarean Section). There were no suggestive group differences regarding any other parameter.

The Developmental Toxicity NOEL is 25 mg/kg/day. The Developmental Toxicity LOEL is 100 mg/kg/day. The only apparent Developmental Toxicity parameter which was suggestive of compound administration was an increase in the number of fetuses and litters in both the mid- (100 mg/kg/day) and high- (250 mg/kg/day) dose groups which were reported to have hydrocephaly or hydrocephaly/dilated cerebral ventricles when compared with this study's control group or the laboratory's historical data.

VI. Core Classification: Supplemental [This may be upgraded to Core Minimum provided the answers to the artificial insemination questions are acceptable.]

Maternal Toxicity NOEL = 100 mg/kg/day
Maternal Toxicity LOEL = 250 mg/kg/day
Developmental Toxicity NOEL = 25 mg/kg/day
Developmental Toxicity LOEL = 100 mg/kg/day

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