

Reviewed by: Roger Gardner *R. G. 12-27-88*
Section 1, Toxicology Branch
Insecticides/Rodenticides (TS 769C)
Secondary Reviewer: *Judith W. Harawich 12/28/88*
Section 1, Toxicology Branch
Insecticides/Rodenticides (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Teratology - Rabbits (Guideline §83-3)

MRID NUMBER: 407171-02

Caswell No.: 640

TEST MATERIAL: Technical grade PCNB with a stated purity 96.0% was used.

SYNONYMS: Pentachloronitrobenzene; Terraclor

STUDY NUMBER(S): 399-070

SPONSOR: Uniroyal Chemical Co.

TESTING FACILITY: International Research and Development Corporation,
Mattawan, MI

TITLE OF REPORT: Developmental Toxicity Study in New Zealand White Rabbits

AUTHOR(S): Keller, K. A.

REPORT ISSUED: June 24, 1988

CONCLUSIONS: Groups of 16 pregnant New Zealand White rabbits were given daily doses of 0 (two groups), 6.25, 12.5, 125 (two groups), and 250 mg PCNB per kg body weight by gavage on gestation days 7 through 19. At the highest dose tested there was mortality, abortions, weight loss during gestation, and decreased food consumption. The only effects observed in the 125 mg/kg/day dose groups were decreased body weight and body weight gain. Group mean maternal body weight was statistically significantly less than control values only during the second of the two trials that were conducted at that dose level. Based on these results, a no-observable-effect level (NOEL) for maternal toxicity was established in the studies at 12.5 mg/kg/day, and the lowest-effect level (LEL) was 125 mg/kg/day.

Based on statistically significantly decreased fetal weights reported in the highest dosed group, the LEL for developmental toxicity was established at 250 mg/kg/day, and the NOEL was found to be 125 mg/kg/day in rabbits.

Core classification: Minimum

I. PROTOCOL

A. Materials

1. Test species: Six-month old female New Zealand White strain rabbits were used. The day the animals were artificially inseminated was designated Day 0 of gestation.

- B. Experimental procedures: The test substance was suspended in aqueous 0.2% carboxymethylcellulose and administered by gavage on Days 7 through 19 of gestation. The report stated that doses were selected on the basis of a preliminary range-finding study (see DER on MRID No. 407171-01). Doses originally selected for the study were 0 (A), 12.5, 125 (A), and 250 mg/kg/day, and because of results discussed below, a second set of doses (0 (B), 6.25, and 125 (B) mg/kg/day) were also tested. The design of the two experiments was described in the report as follows:

<u>Group number *</u>	<u>Dose (mg/kg/day)</u>	<u>Number of animals</u>
1	Control A	16
2	Control B	16
3	6.25	16
4	12.5	16
5	125 (A)	16
6	125 (B)	12
7	250	16

* For purposes of this review.

- C. Maternal observations: Each doe was observed twice daily for occurrence of toxic signs and morbidity. Body weight determinations were made on days 0, 7, 13, 20, 24 and 29 of gestation, and food consumption was measured for days 7-13, 13-20, 7-20, 20-29, and 0-29 of gestation.

Surviving rabbits were weighed and sacrificed on day 29 of gestation. Internal organs were examined for gross lesions, and the gravid uterus was removed and weighed. The numbers of corpora lutea and implantation sites were noted.

Does dying prior to the end of the study were examined for gross pathological changes, and the pregnancy status of these animals was noted. Uteri from nongravid animals were stained with ammonium sulfide to detect implantations.

- D. Litter observations: The number of implantation sites, live and dead fetuses, and resorptions were counted.
- E. Fetal observations: Fetuses were individually weighed and examined for external abnormalities. The fetuses were then internally sexed, and their viscera were examined for variations and malformations. The brain was examined through a transverse section between the

E. Fetal observations (continued)

parietal and frontal bones of the unfixed fetal head. Fetuses were then fixed in ethanol and emaciated in potassium hydroxide. The skeletons were then stained with alizarin red S for examination.

F. Evaluation of observations: All external, visceral, and skeletal alterations were classified into two categories defined in the report as follows:

Malformations are those structural anomalies that alter body conformity, disrupt or interfere with body function, or are generally thought to be incompatible with life. Specific examples of processes that result in maldevelopment include marked/severe mishapening, assymetry or irregularity of structure brought about by fusion, splitting, disarticulation, malalignment, hiatus, enlargement, lengthening, thickening, thinning, or branching. Absence (agenesis) of parts or whole structures is also considered a malformative process.

Developmental variations are those alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity, representing slight deviations from normal. Most examples of alterations placed in the variant category are minor variations in size and form of normally present ossification centers...Also included in this category of variations are slight mishapening or misalignment of structures, processes involving continued development (bilateral skeletal centers not yet fused, incomplete maturation of renal papillae, presence of vestigial structures, etc) and development of extra ossification sites.

Statistical comparisons were made between each treatment group and its appropriate concurrent control group (i.e., Control A was used with the 12.5, 125 (A), and 250 mg/kg/day groups and Control B was used with the 6.25 and 125 (B) mg/kg/day groups).

Differences were considered to be statistically significant at $p < 0.05$.

Statistical analyses were used in the report as follows:

<u>Observation</u>	<u>Test for Linear Trend</u>	<u>Between Groups</u>
Male to female sex ratios	None	Chi square (2 X 2) with Yates' correction
Proportions of litters with malformations or variations	None	Fisher's Exact test

F. Evaluation of observations (continued)

<u>Observation</u>	<u>Test for Linear Trend</u>	<u>Between Groups</u>
Proportions of resorptions, dead fetuses, and postimplantation losses	None	Mann-Whitney U test
Means for numbers of: Corpora lutea Total implantations Live fetuses Gravid uterine weights Fetal body weights Maternal food consumption	None	One-way classification of variance, Bartlett's test for homogeneity of variances, Dunnett's multiple comparison

II. REPORTED RESULTS

A. Maternal observations: Mortality and pregnancy status of the does is summarized as follows:

<u>Parameter</u>	<u>Dose (mg/kg/day)</u>						
	<u>Study A</u>				<u>Study B</u>		
	<u>0</u>	<u>12.5</u>	<u>125</u>	<u>250</u>	<u>0</u>	<u>6.25</u>	<u>125</u>
Number inseminated	16	16	16	16	16	16	12
Number not pregnant	1	0	3	1	1	3	1
Number pregnant	15	16	13	15	15	13	11
Deaths	0	1	2 *	5	0	0	0
Abortions	2	2	5 *	5 **	0	0	1
Total resorptions	1	0	0	0	0	0	0
Delivered	1	0	1	0	1	0	0
Number of litters	11	13	7	6	14	13	10

*Includes the two females that aborted and died.

**One of these also died after aborting.

The deaths occurred during days 19 through 28 of gestation. According to the report, gross necropsy findings (mucoid material in the intestinal tract or hemorrhage of the trachea with red discoloration and/or "consolidation" in the lungs) indicated that two deaths were the result of mucoid enteritis (125 (A) mg/kg/day dose group) and three resulted from pneumonia (250 mg/kg/day dose group). The remaining mortalities could not be associated by the investigators with a cause.

Abortions were noted during days 23 through 28 of gestation.

A. Maternal observations (continued)

The incidences of mucoid stool, absence of stool, and anogenital staining were the only signs noted in the report. The incidences of these observations is summarized as follows:

Observation	Dose (mg/kg/day)							
	Study A				Study B			
	0	12.5	125	250	0	6.25	125	
Number examined	16	16	16	16	16	16	12	
Mucoid stool	0	0	1	6 *	0	0	0	
Absence of stool	4	3	8	11 *	2	1	1	
Anogenital staining	3	8	9	10 *	2	3	3	

* Statistically significantly different from control, $p < 0.05$;
Fisher's Exact test.

The report noted that animals in the 125 (A), 125 (B), and 250 mg/kg/day dose groups exhibited weight losses during the dosing period. Overall weight loss results are summarized as follows:

Dose (mg/kg/day)	Mean weight (kg) on gestation†		Weight change (kg) for	
	Day 0	Day 29††	Days 7-20	Days 0-29 ††
Study A				
0	4502	4231	-55	-251
12.5	4452	4152	91	-261
125	4449	4101	-192	-290
250	4432	3693	-523	-706
Study B				
0	4356	4160	151	-230
6.25	4226	3917	145	-309
125	4182	3844	-42	-322

†The report did not include statistical analyses of these results.

††Body weights at day 29, corrected for gravid uterine weight.

†††These values were calculated from group mean body weights.

No statistical analyses were conducted on these data.

Food consumption results are summarized as follows:

A. Maternal observations (continued)

Dose (mg/kg/day)	Mean feed consumption (g/kg/day)				
	7-13	13-20	7-19	20-29	7-29
Study A					
0	30.2	23.7	26.5	18.7	24.6
12.5	31.8	26.0	27.6	18.3	25.7
125	31.5	19.2	25.3	22.4	29.2
250	22.1	5.9 **	14.6 *	3.7 *	14.2
Study B					
0	41.4	38.3	39.4	23.6	33.5
6.25	38.1	32.0	34.6	24.8	30.7
125	32.9 **	24.5 *	28.7 **	27.0	28.9

* Statistically significantly different from control, $p < 0.05$
 ** Statistically significantly different from Control, $p < 0.01$

B. Litter observations: The report summarized these observations (means per litter) follows:

Observation	Dose (mg/kg/day)						
	Study A				Study B		
	0	12.5	125	250	0	6.25	125
Number of litters	11	13	7	6	14	13	10
Live fetuses							
Males*	3.4	2.8	4.3	2.5	2.6	3.7	3.7
Females*	3.7	3.7	2.7	2.2	3.2	4.1	3.1
Total**	6.7	6.5	6.9	4.2	5.9	7.8	6.8
Corpora lutea	15.2	14.1	13.3	12.0	16.2	14.3	14.3
Implantations	7.2	7.4	7.9	6.0	7.8	8.7	7.2
Postimplantation loss	0.5	0.9	1.0	1.8	1.9	0.9	0.4
Fetal weight (g)							
Both sexes combined	41.4	39.1	40.3	28.4 †	44.7	42.6	47.2
Gravid uterine weight (g)	441.6	358.7	433.9	235.7	358.0	487.4	429.3

* Calculated from the total number of male and female fetuses reported and the number of litters examined in the group.

** Listed here as reported.

† Statistically significantly different from control A, $p < 0.05$.

C. Fetal observations: The report noted that there were no statistically significantly increased incidences of malformations in fetuses from treated groups. There were also no treatment-related incidences of developmental variations in fetuses examined in the study. The results as they were summarized in the report are included in Addenda A and B below.

III. DISCUSSION

A. Investigators' conclusions: The report concluded:

Excessive maternal toxicity, in terms of an increased incidence of mortality, abortion and certain stool abnormalities as well as adverse effect on weight gain and food consumption, was noted at the 250 mg/kg/day dosage level. These effects were also observed at the 125 mg/kg/day dosage level, but, with the exception of increased weight loss, did not occur consistently in the two groups that received this dosage level. Developmental toxicity (reduced fetal body weight) was present at the 250 mg/kg/day dosage level. There were no other effects on fetal development, including teratogenicity.

In conclusion, the no observable effect level in regard to developmental toxicity was determined to be 125 mg/kg/day.

B. Reviewer's discussion: The report did not include statistical analysis of maternal body weight results, so an analysis was independently conducted. The individual body weights were first analyzed by one-way analysis of variance and Bartlett's test for homogeneity of variance. The results of those analyses showed that the data required transformation, and a log transformation was used. An analysis of variance was conducted on the transformed data, and appropriate concurrent control groups were compared with each treatment group using a t test. Statistically significant differences are summarized with untransformed group means as follows:

Dose (mg/kg/day)	Day 13	Group mean body weight (g) *			
		Day 20	Day 24	Day 29	Day 29**
Experiment A					
0	4586	4539	4649	4547	4231
12.5	4630	4609	4607	4510	4152
125	4564	4428	4433	4489	4101
250	4409	4034 ††	4003 ††	3929 ††	3693 ††
Experiment B					
0	4523	4591	4623	4518	4160
6.25	4372	4408	4465	4404	3917
125	4312	4248 †	4343	4273	3844 †

* Group means are not transformed.

** Adjusted by subtracting the weight of the gravid uterus.

† Statistically significantly different from controls (p<0.05). t test.

†† Statistically significantly different from controls (p<0.01). t test.

B. Reviewer's discussion (continued)

Because there were so few does and litters available for evaluation in the first study, results from the 125 mg/kg/day dose group can not be compared reliably with those from the 125 mg/kg dose group in the second experiment. The 125 mg/kg/day dose group in Experiment B indicated that body weight and food consumption were significantly reduced which suggested that 125 mg/kg/day is the lowest effect level for maternal toxicity. Based on body weight and food consumption results from both experiments, the no-observed-effect level (NOEL) for maternal toxicity is 12.5 mg/kg/day.

Fetal effects (reduced body weight) were observed only at the 250 mg/kg/day dose level (highest dose tested) along with excessive maternal toxicity (mortality, abortions, reduced body weight and decreased food consumption). The NOEL for fetal effects was 125 mg/kg/day. No increased incidences of teratogenic effects were observed in PCNB treated animals.

ADDENDUM A

Incidence of Malformations in
Fetuses from Rabbits Treated with
Pentachloronitrobenzene

TABLE 5. Summary of the Incidence of Fetal Malformations

	PCNB (mg/kg/day)		
	0 (Control A)	0 (Control B)	6.25
No. of litters examined:	17 ^a	15 ^a	13
No. of fetuses examined externally:	94 ^b	90 ^b	101
No. of fetuses examined viscera:	94 ^b	90 ^b	101
No. of fetuses examined skeletally:	94 ^b	90 ^b	101

Malformations Observed:

	No. of fetuses (No. of litters)		
Microcephaly:	2 (1)		
Encephalocele:	1 (1)		
Spina bifida:	1 (1)		
Gastroschisis:			
Omphalocele:	1 (1)		
Syndactyly:			
Malformed brain:			
Bulbous aortic arch:	2 (1)		
Coarction of aortic arch:			
Stenosis of aortic arch:			
Stenosis of ascending aorta:			
Bulbous pulmonary trunk:			
Stenosis of pulmonary trunk:			
Pulmonary hypoplasia:			
Stenosis of ductus arteriosus:			
Interventricular septal defect:			
Right subclavian arclses from pulmonary trunk:	1 (1)		
Malformed heart:			
Adrenal agenesis:			
Distended intestines:			
Malpositioned kidney:			
Fused kidneys:	1 (1)		
Gallbladder agenesis:	1 (1)		
Fused skull bones:			
Malformed skull bones:	3 (2)		
Micrognathia:			
Bent scapula:	1 (1)		
Vertebral anomaly with or without associated rib anomalies:	1 (1)		
Rib anomaly:			
Interrupted ossification of rib:	1 (1)		
Sternoschisis:	1 (1)		
Total fetuses (litters) with malformations:	4 (3)	4 (3)	9 (6)

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Values from the treated groups did not differ significantly from those of the concurrent control group; p>0.05
^aincludes litters that delivered, aborted or died on gestation day 28 or 29
^bincludes nonviable fetuses

TABLE 5. Cont. Summary of the Incidence of Fetal Malformations

	PCNB (mg/kg/day)		No. of fetuses (No. of litters)
	125(A)	125(B)	
No. of litters examined:	10 ^a	10	6
No. of fetuses examined externally:	73 ^b	68	28 ^b
No. of fetuses examined viscerally:	73 ^b	68	28 ^b
No. of fetuses examined skeletally:	73 ^b	68	28 ^b
Malformations Observed:			
Microcephaly:			
Encephalocele:			
Spina bifida:			
Gastroschisis:			
Omphalocele:			
Syndactyly:	1 (1)		
Malformed brain:			
Bulbous aortic arch:			
Coarction of aortic arch:			
Stenosis of aortic arch:			
Stenosis of ascending aorta:			
Bulbous pulmonary trunk:			
Stenosis of pulmonary trunk:			
Pulmonary hypoplasia:			
Stenosis of ductus arteriosus:			
Interventricular septal defect:			
Right subclavian arises from pulmonary trunk:			
Malformed heart:			
Adrenal agenesis:			
Distended intestines:			
Malpositioned kidney:			
Fused kidneys:			
Gallbladder agenesis:			
Fused skull bones:	1 (1)		
Malformed skull bones:			
Micromethia:		1 (1)	
Bent scapula:			
Vertebral anomaly with or without associated rib anomalies:			
Rib anomaly:		1 (1)	
Interrupted ossification of rib:	1 (1)	1 (1)	
Sternoschisis:			
Total fetuses (litters) with malformations:	3 (3)	2 (2)	0 (0)

Values from the treated groups did not differ significantly from those of the concurrent control group; p>0.05
^aIncludes litter(s) that delivered, aborted or died on gestation day 28 or 29
^bIncludes nonviable fetus(es)

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ADDENDUM B

Incidence of Variations in
Fetuses from Rabbits Treated with
Pentachloronitrobenzene

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TABLE 6. Summary of the Incidence of Fetal Developmental Variations

	PCNB (mg/kg/day)		
	0 (Control A)	0 (Control B)	6.25
No. of litters examined:	12 ^a	15 ^b	13
No. of fetuses examined externally:	94 ^b	90 ^b	101
No. of fetuses examined visceraally:	94 ^b	90 ^b	101
No. of fetuses examined skeletally:	94 ^b	90 ^b	101

Developmental Variations Observed:

Thickened body:
 Carpal flexure:
 Tarsal flexure:
 Short tail:
 Hemorrhagic irides:
 Left carotid arises from the innominate:
 Azygous lobe of lung absent:
 Retroesophageal aortic arch:
 Gallbladder smaller than normal:
 Bont hyoid arch(es):
 Hyoid body unossified:
 Missing skull bones:
 Accessory skull bones:
 Supraoccipital reduced in ossification:
 27 presacral vertebrae:
 24 presacral vertebrae:
 13th rudimentary rib(s):
 Greater than 12 pairs of full ribs:
 7th cervical rib:
 Spherical enlargement of rib(s):
 Missing ribs:
 Sternebrae #5 and/or #6 unossified:
 Fused and/or misaligned sternbrae:
 Extra sternbral site of ossification:
 Pubic bones unossified:
 Tail unossified:

	No. of fetuses (No. of litters)		
	1 (1)	1 (1)	1 (1)
	3 (2)	2 (1)	8 (5)
	5 (2)	3 (3)	3 (2)
	1 (1)	1 (1)	1 (1)
	1 (1)	3 (2)	4 (3)
	2 (1)	1 (1)	2 (2)
	35 (7)	18 (6)	31 (9)
	9 (6)	15 (9)	25 (10)
	55 (8)	36 (10)	46 (13)
	1 (1)	2 (1)	5 (3)
	1 (1)	1 (1)	1 (1)
	10 (5)	3 (2)	23 (8)
	3 (3)	2 (2)	1 (1)
		1 (1)	
Total fetuses (litters) with variations:	74 (12)	59 (14)	82 (13)

^aincludes litter(s) that delivered, aborted or died on gestation day 28 or 29
^bincludes nonviable fetus(es)

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TABLE 6. Cont. Summary of the Incidence of Fetal Developmental Variations

	PCNB (mg/kg/day)	
	125(A)	250
No. of litters examined:	10 ^a	10
No. of fetuses examined externally:	73 ^b	68
No. of fetuses examined visceraally:	73 ^b	68
No. of fetuses examined skeletalily:	73 ^b	68

Developmental Variations Observed:

	No. of fetuses (No. of litters)	
Thickened body:	1 (1)	
Carpal flexure:		
Tarsal flexure:	1 (1)	
Short tail:		
Hemorrhagic iris:		
Left carotid arises from the innominate:	2 (2)	2 (2)
Azygos lobe of lung absent:	2 (1)	
Retrosophageal aortic arch:	4 (2)	
Gallbladder smaller than normal:	2 (1)	1 (1)
Bent hyoid arch(es):	5 (4)	2 (1)
Hyoid body unossified:	1 (1)	
Misshaped skull bones:		
Accessory skull bones:		
Supraoccipital reduced in ossification:	14 (4)	20 (5)
27 presacral vertebrae:		
24 presacral vertebrae:	14 (8)	13 (5)
15th rudimentary rib(s):	31 (8)	37 (7)
Greater than 12 pairs of full ribs:	1 (1)	1 (1)
7th cervical rib:		
Spherical enlargement of rib(s):		
Misshaped ribs:	15 (6)	1 (1)
Sternebra(e) #5 and/or #6 unossified:		5 (2)
Fused end/or misaligned sternbrae:		1 (1)
Extra sternbral site of ossification:	11 (2)	
Pubic bones unossified:	18 (4)	
Tail unossified:		
Total fetuses (litters) with variations:	59 (10)	54 (8)

Total fetuses (litters) with variations:

^aincludes litter(s) that delivered, aborted or died on gestation day 28 or 29
^bincludes nonviable fetuses

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