DEC 15 1988

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

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DEC 15 '88

MEMORANDUM:

SUBJECT: Propachlor - Review of Studies Listed in TB Memo of 6/16/88

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

TO: Jim Thompkins
Product Manager (41)
Registration Division (TS-767C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/5/88
Tox. Branch II, Section II
Health Effects Division (TS-769C)

THRU: James N. Rowe, Ph.D. James N. Rowe 12/6/88
Tox. Branch II, Acting Section II Head
Health Effects Division (TS-769C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 12/9/88
Acting Chief, Tox. Branch II
Health Effects Division (TS-769C)

Registrant: Monsanto Company
Chemical: Propachlor; 2-chloro-N-(1-methylethyl)-N-phenylacetamide
Project No. 8-0795
Caswell No. 194
Record No.: 223957
Action Requested: Review toxicology studies.

Background: In a previous TB memorandum (June 16, 1988), data were forwarded to TB for a brief assessment in order to determine whether a Section 18 could be granted for the use of Propachlor on dry bulb onions in New York State. Full reviews of those data were not prepared at that time.

These studies have been reviewed and the DER's are attached. Additionally, during the review of these data, it was discovered that the mouse oncogenicity study had not been evaluated and, although it was not a part of the current bean, has been evaluated and the DER is included with the others. A summary of each data review is provided below (A). The supplemental data on the rabbit teratology study has also been reviewed and is commented on below (B).

A. Summaries of the DER 's on the previously-submitted studies on Propachlor.

1. Combined chronic/oncogenicity study in rats - HL-83-350/241-160, dated August 14, 1987. There were no effects observed in any of the parameters monitored following exposure to Propachlor at dose levels of 10, 50, and 500 ppm in the diet for 104 weeks, with the possible exception of a slight increase in the incidence of thyroid and ovarian neoplasia. Historical control tumor incidence data should be requested. The doses appear to be too low to adequately assess the chronic/oncogenic potential of Propachlor.

2. Three-month feeding study in mice - ML-81-72, dated October 9, 1984. This study was subjected to a data audit, but the issues raised by it have not been addressed to date. There appears to be no NOEL for liver effects observed after exposure to Propachlor at dose levels of 500, 1500, and 5000 ppm in the diet for 3 months. Because there is an oncogenicity study in mouse available, there is no need to repeat this 3-month study at this time.

3. Acute In Vivo rat bone marrow cytogenetics assay - SR-84-180, dated August, 1985. Justification for the dose levels used is required, along with clarification of whether the test material used was the technical grade of Propachlor.

4. CHO/HGPRT gene mutation assay - ML-84-237, dated August 2, 1985. Under the conditions of this assay, Propachlor induced a concentration-dependent increase over the range 30-50 ug/ml to over a doubling of the solvent control mutant frequency at the HGPRT locus of the Chinese hamster ovary (CHO) cells, with activation. No apparent increase was obtained without activation.

5. Unscheduled DNA synthesis in primary rat hepatocyte cultures - SR-84-239. Under the conditions of this assay, Propachlor was not shown to be genotoxic in the in vitro rat hepatocyte DNA repair assay at concentrations up to 25 ug/ml (higher levels cytotoxic); however, this assay is unacceptable pending clarification of whether the test material used was the technical grade.

6. Chromosomal aberration frequencies/induction in Chinese hamster ovary cells - MSL-6930, dated June 23, 1987. Propachlor induced a clastogenic effect under metabolic activation conditions and was found to be negative for induction of chromosomal aberrations in CHO cells without metabolic activation.

7. Oncogenicity study in mice - HL-83-349, dated January 19, 1987. Under the conditions of the study, Propachlor showed no oncogenic potential at dose levels up to 500 ppm. However, the dose levels used do not appear to have been high enough to adequately assess the oncogenic potential of Propachlor.

8. Rabbit teratology study -IR-82-224, dated May 8, 1987. The NOEL for maternal toxicity is 50 mg/kg (HDT). No NOEL can be set for developmental toxicity since in addition to the apparent disease in the animals and an increased pre-implantation loss in all treated groups (indicative of animal husbandry problems), there was an insufficient number of litters at the low-dose level, which precludes the setting of the NOEL.

B. Data Call-In Submission - Rabbit Teratology Study (IR-82-224, EPA Accession No. 255758) - study previously evaluated (TB memo dated 12/24/86) and classified as supplementary, pending submission of definitions for the major vessel variations, as well as for variations versus malformations, additional historical control data, individual maternal necropsy data, and individual fetal variation data. These have been submitted and are adequate.

This reviewer (LLT - not the original reviewer) has considered the recently submitted tables on individual maternal necropsy and summary antemortem observations, individual fetal variations, revised historical control tables, as well as the original report and TB review and has the following comments with regard to the rabbit teratology study.

DEFINITIONS: a) major vessel variations - include left carotid arising from the innominate, no innominate and retroesophageal right subclavian, all common heart variations in this species and strain; b) malformations - those structural anomalies that alter general body conformity, disrupt or interfere with body function, or are generally thought to be incompatible with life (marked/severe misshapening, asymmetry or irregularity of structure brought about by fusion, splitting, disarticulation, malalignment, hiatus, enlargement, lengthening, thickening, thinning, or branching; absence (agenesia) of parts or whole structures); c) variations - alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity, representing slight deviations from normal (minor variations in size and form of normally present ossification centers. Since these are evaluated on a precise day of development, some variation is expected, which is related to when conception and implantation actually occurred: differences in the pattern of ossification (retardation or acceleration), 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.

CLARIFICATION: a) tabulation - refers to numbers of fetuses/litters affected; b) when a malformation exists in a particular site, a variation in the same site is also tabulated; c) where two or more variations of the same type or structure exist in the same specimen, all are counted, with a single exception: a unilateral rudimentary rib is not tabulated when an extra rib is presented; d) the kidney was evaluated according to criteria described by Woo and Hoar (Teratology 6, 191-196 (1972)), and only grade zero lesions of the renal pelvis and papilla (absent papillae) were tabulated as variations; e) dilatation of the ureter was also included in the tabulation, whether or not it accompanied renal lesions.

The number of deaths during the study was not different among the groups, although the number of does delivering litters was different.

	# pregnant	# litters	# aborted	# dead
Control	14/16	12	0	2
Low	12/16	8	2	3
Mid	14/16	11	1	3
High	16/16	14	0	2

The treated does appeared to gain weight and/or maintained their body weight better than the control animals. The overall body-weight gain (Day 0 to Day 29 (adjusted for uterine weight)) was negative in the controls and positive in all treated groups.

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	Days 0-7	Days 7-19	Days 19-25	Days 19-29	Days 0-29	Days 0-29A
Control	122	134	82	107	363	-74
Low	137	93	95	154	384	6
Mid	124	61	21	112	297	5
High	153	138	85	116	407	66

There was an increase in pre-implantation loss in all treated groups, which also exceeded the historical control (2.9) value, as well as an increase in early resorptions (all treated groups compared to control), although the mid-dose group showed the greatest increase.

	Pre-implantation loss			Resorptions or late deaths/litter			
	# ± SD	%		#		%	
				L	E	L	E
Control	2.7 ± 1.4	23.4	Control	0.3	0.3	3.1	2.8
Low	4.8 ± 4.6	31.8	Low	0.0	0.4	0.0	7.8
Mid	5.5 ± 3.0	40.7	Mid	0.0	1.4	0.0	25.5
High	6.0 ± 4.5	42.1	High	0.2	0.9	3.2	15.4

Post-implantation loss (early + late resorptions and non-viable fetuses) was also increased in the treated groups (mid dose displayed the highest increase).

Post-implantation loss	
# ± SD	%
0.6	5.9
0.4	7.8
1.5*	27.3*
1.1	18.7

*includes one non-viable fetus

Note: The equation presented in the original TB review (page 5) of this study for % Post Implantation Loss is incorrect; it should be:

$$\% \text{ post implantation loss} = \frac{\text{total implantations} - \text{total viable fetuses}}{\text{total implantations}} \times 100$$

The number of live fetuses was decreased at the mid- and high-dose levels, and both of these dose levels had one doe with 100% resorptions.

	Live fetuses/doe	
	#	%
Control	8.0	94
Low	7.9	92
Mid	5.2	73
High	5.9	74

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The number of corpora lutea/doe was unaffected by treatment, while the number of implants/doe was slightly decreased in the mid- and high-dose groups.

	corpora lutea/doe	# implants/doe
Control	11.4	8.6
Low	13.0	8.3
Mid	12.7	6.6
High	13.0	7.0

There were no maternal toxic effects reported at any dose level. The clinical observations and necropsy findings (nasal and ocular discharge, lung congestion, foci) suggest a possible infection (or dosing problem) in some of the animals. Additionally, although there was no significant increase in malformations/ variations in any of the treated groups, only the treated groups displayed malformations.

DISCUSSION

The NOEL for maternal toxicity is 50 mg/kg (HDT). No NOEL can be set for developmental toxicity since, in addition to the apparent disease in the animals and the increased pre-implantation loss in all treated groups (indicative of an animal husbandry problem), there was a low number of litters at the low-dose level.

Although the authors stated that 5 mg/kg was the NOEL, the lack of a sufficient number of does (litters) at this dose level precludes setting the NOEL at this (5 mg/kg) dose.

CONCLUSION

This study is classified as Supplementary; no NOEL was observed for developmental toxicity. In addition, the high incidence of nasal discharge and congested lungs leads to questions about the health status of the test animals. This study should be repeated using at least one dose level below 5 mg/kg in order to determine the developmental toxicity NOEL, since the number of litters at the 5 mg/kg level in the current study are too few to make any definitive statement on whether an effect was obtained at this level.

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Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe, 12/6/88
Acting Head, Section II, Tox. Branch II (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Combined chronic/oncogenic - rat TOX. CHEM. NO.: 194 (CASWELL #)

MRID NO.: 404731-01

TEST MATERIAL: 2-chloro-N-(1-methylethyl)-N-phenylacetamide

SYNONYMS: Propachlor; Ramrod®

STUDY NUMBER(S): HL-83-350/241-160

SPONSOR: Monsanto Chemical Company

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Combined Chronic Toxicity and Oncogenicity Study in Rats (Propachlor)

AUTHOR(S): N. Nicki Hamada, Ph.D. and Susan G. Landes, B.S.

REPORT ISSUED: August 14, 1987

CONCLUSIONS: There were no effects observed in any of the parameters monitored following exposure to Propachlor at dose levels of 10, 50, and 500 ppm (diet) for 104 weeks, with the possible exception of a slight increase in the incidence of thyroid and ovarian neoplasia. Before a final assessment can be made, historical control incidence of granulosa/theca cell tumors (ovary) and "C" cell adenoma/carcinoma (thyroid) observed at the testing facility should be submitted for review. The doses used in this study do not appear to have been high enough to adequately assess the chronic toxicity or the oncogenic potential of Propachlor.

Classification: core-supplementary, pending submission of historical control data on the incidence of granulosa/theca cell tumors of the ovary and "C" cell adenoma/carcinoma of the thyroid. If these data support the study conclusion that the incidence of these lesions in the current study is within the historical control range, this study will remain "supplementary", since an MTD was not attained.

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A. MATERIALS:

1. Test compound: Propachlor; Description: a light brown, sandy to flaky powder containing granular material of the same color; Batch #: Lot # MDRF 1114B; Purity: 96.1 %.

2. Test animals: Species: Rat ; Strain: Sprague-Dawley CD®-Crl:CD(SD)BR; Age: approximately 6 wks old; Weight: males: 206-261 g, females: 129-184 g; Source: Charles River Breeding Laboratories, Inc., Portage, MI.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Group (ppm)	Dose in diet (ppm)	MALES	FEMALES
1 Control	0	60	60
2 Low	10	60	60
3 Mid	50	60	60
4 High	500	60	60

2. Diet preparation

Fresh diets were mixed twice weekly. Homogeneity and stability diet analyses were determined prior to study initiation. Samples from the prepared test diets were analyzed retrospectively for concentration of test material (all levels) from the following intervals: weeks 0, 1-12, 15, 20, 23, 28, 31, 36, 39, 44, 47, 52, 55, 60, 63, 68, 71, 76, 80, 84, 88, 92, 96, 100, and 104.

Note: It was recognized early in the study preparation that the analytical recovery of Propachlor from the 7-day rodent chow was consistently low. Investigations were performed to determine the reason(s) for this finding. One study indicated that the low Propachlor recovery was the result of low extraction efficiency rather than degradation or volatilization. A second study reported that Propachlor is stable on rodent diet for a period of time adequate for the proper conduct of this study.

3. Animals, housed singly, received food and water ad libitum throughout the study.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

[redacted]

Survival - cumulative survival data through week 104 were analyzed using the National Cancer Institute Package (Thomas, Breslow, and Gart, 1977); trend analysis of survival: evaluated at the 5% one-tailed probability level.

Absolute body weights (at week 104), growth rates (Rao's growth parameters), total food consumption (weeks 1-50 and 0-104), clinical pathology data (except leukocyte differentials, erythrocyte morphology, and urinalysis), and organ weight data of the control group were compared to treated groups of the same sex. Analysis of these data was performed as diagrammed in Figure 1 (Attached).

Tests for homogeneity of variance and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. compound-treated group mean comparisons of the data were evaluated at the 5% two-tailed probability level.

5. Quality Assurance - A quality assurance statement was provided.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of mortality and moribundity; for toxic effects, once daily. Detailed physical examinations were performed weekly and included observation of appearance, behavior, changes in excreta, toxicologic and pharmacologic signs, and tissue mass development. Individual body weights were recorded at study initiation, and body weight and food consumption measurements were recorded weekly for weeks 1-14, and monthly thereafter. At 53 weeks, 10 animals/group were sacrificed.

Results:

Toxicity/Mortality (survival)

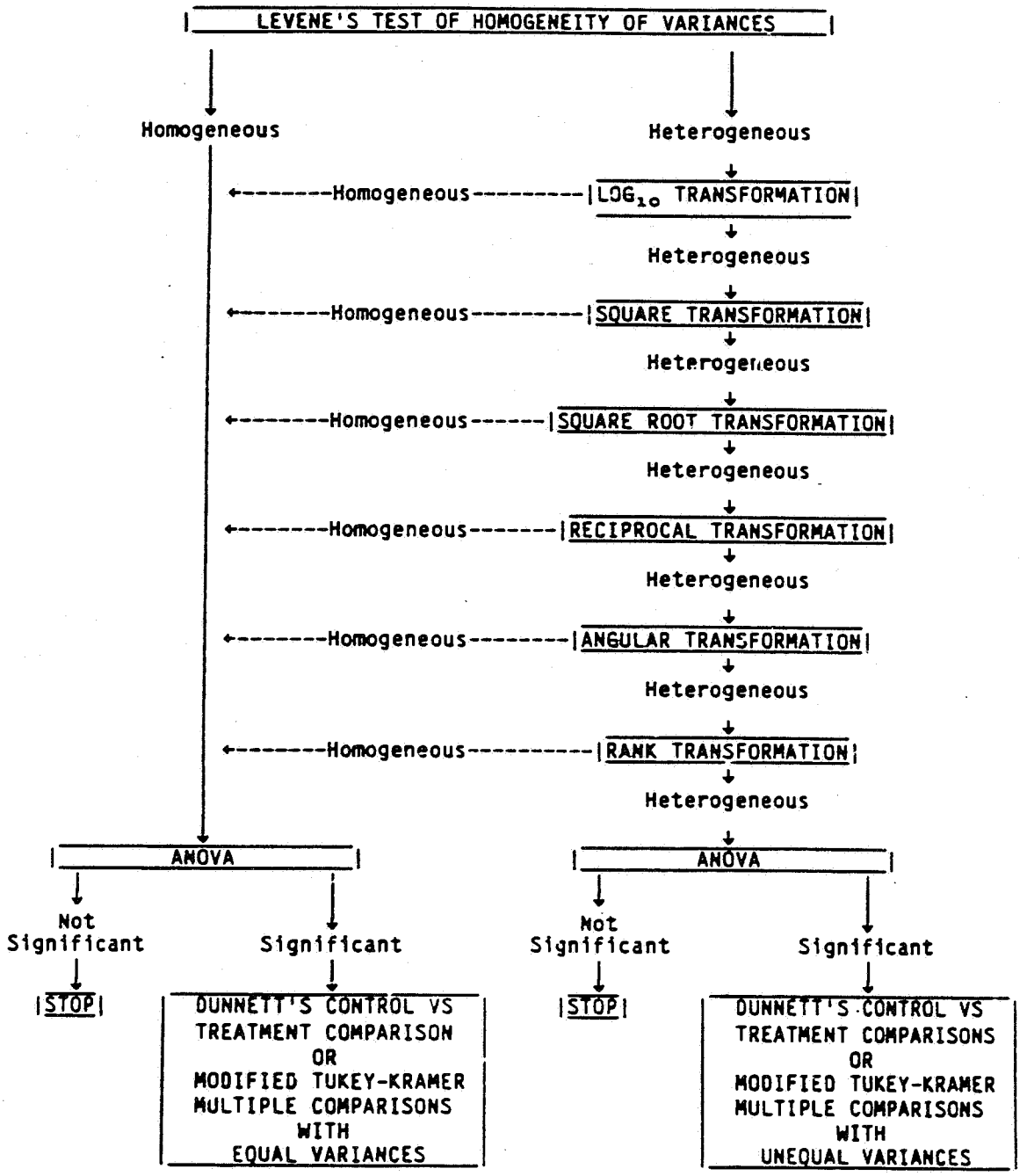
There were no significant differences reported in survival among the treated groups of animals compared to their respective controls throughout the study. The number of animals surviving to week 104 is listed below.

SURVIVAL (%)

	MALES	FEMALES
Control	29/50 (58)	24/48 (50)
Low	35/50 (70)	31/50 (62)
Medium	31/50 (62)	28/49 (57)
High	31/50 (62)	34/50 (68)

Seventy-nine male and eighty-six female rats died spontaneously/sacrificed moribund during the course of the study; the most common cause of death

Figure 1


LEVENE'S TEST OF HOMOGENEITY OF VARIANCES
Homogeneous
Heterogeneous
Homogeneous
LOG10 TRANSFORMATION
Heterogeneous
Homogeneous
SQUARE TRANSFORMATION
Heterogeneous
Homogeneous
SQUARE ROOT TRANSFORMATION
Heterogeneous
Homogeneous
RECIPROCAL TRANSFORMATION
Heterogeneous
Homogeneous
ANGULAR TRANSFORMATION
Heterogeneous
Homogeneous
RANK TRANSFORMATION
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ANOVA
ANOVA
Not Significant
Significant
Not Significant
Significant
STOP
DUNNETT'S CONTROL VS TREATMENT COMPARISON OR MODIFIED TUKEY-KRAMER MULTIPLE COMPARISONS WITH EQUAL VARIANCES
STOP
DUNNETT'S CONTROL VS TREATMENT COMPARISONS OR MODIFIED TUKEY-KRAMER MULTIPLE COMPARISONS WITH UNEQUAL VARIANCES

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was reported as pituitary neoplasia.

	Cause of death/morbidity							
	MALES				FEMALES			
	C	L	M	H	C	L	M	H
# unscheduled deaths	22	17	20	20	26	21	22	17
pituitary neoplasia	14	10	7	9	17	13	15	10
undetermined	2	3	7	3	2	3	4	1
all other causes	6	4	6	8	7	5	3	6

Body Weight

No differences were reported in absolute body weight in the males, but group mean body weights in the mid-dose females were reported to be significantly lower* than the control females at week 2; the mid- and high-dose at weeks 4, 6, and 14; and in the high-dose at weeks 12 and 22. Growth rates through week 50 were comparable among the groups of both sexes, and mean body weight changes through week 104 were reported as comparable also. An examination of the female body-weight data indicates that the mid- and high-dose groups displayed a slightly lower body weight at various times during the study (see table below), but these decreases were never more than 5% below the control value and were never sustained.

Week	% Control - Females		
	Low	Mid	High
0	98.7	98.2	100.0
1	98.4	97.7	99.5
2	97.4	97.0*	97.4
4	97.7	96.2*	96.7*
6	97.6	96.2*	96.2*
8	97.4	97.4	96.8
10	98.4	97.9	96.8
12	97.8	96.8	96.4*
13	97.3	96.8	96.2
14	98.1	96.6*	96.0*
22	97.6	97.3	95.2*
42	100.2	99.9	99.1
74	97.7	95.8	96.6
86	95.6	96.4	95.9
90	93.3	98.0	97.0
104	97.5	105.0	102.1

Body-weight changes for three intervals are listed below for the females.

Interval	Control	Low	Mid	High
Weeks 0-13	129.7	124.1	123.2	118.9 (91.7% C)
Weeks 0-90	289.9	262.5	283.9	276.6
Weeks 0-104	254.2	245.9	278.0	262.8

The high-dose group showed a lower (8%) body-weight gain during the first 90 days of the study compared to control.

Food Consumption and Compound Intake

No differences in food consumption were reported throughout the study. The overall mean compound consumption (based on group mean food intake, group mean body weight, and nominal concentrations in the diet) were listed as follows:

	Compound Consumption (mg/kg/day)		
	Low	Mid	High
MALES	.48	2.39	23.88
FEMALES	.60	3.04	30.05

2. Ophthalmological examinations

Ophthalmological examinations were performed on all animals (both sexes) prior to study initiation and during weeks 26, 52, and 104.

Results

No compound- or dose-related ophthalmic abnormalities were reported at weeks 26 and 52. Cataracts (diffuse or focal) were listed for all male groups and in the control, low- and mid-dose females at week 104. These were not dose-related.

3. Clinical Pathology

Blood was collected from 10 animals/sex/group (fasted overnight), as was urine during weeks 26, 52, 78, and 104.

a. Hematology

The following parameters (required for chronic studies) were measured in all animals (* performed only in the control and high-dose groups).

Hematocrit (HCT)	Leukocyte differential count*
Hemoglobin (HGB)	Erythrocyte count (RBC)
Platelet count	Leukocyte count (WBC)

b. Clinical Chemistry

The CHECKED (X) parameters were measured in all animals.

	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*

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X	Phosphorus*	X	Cholesterol*
X	Potassium*	X	Globulin
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase*		Serum protein electrophoresis
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transpeptidase		
	glutamate dehydrogenase		

* Required for chronic studies

c. Urinalysis

The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen
	Osmolality		

* Required for chronic studies

Results: No consistent changes in clinical pathology parameters were reported. With regard to clinical chemistry data, the blood urea nitrogen value was higher in the high-dose females at week 52 and lower in the low-dose males at week 78, compared to their respective controls. Total protein was lower in the high-dose males at termination, which the authors attribute to an increase in the control value. Neither change was considered (by the authors) to be of biological importance. There were large differences observed in the levels of alanine aminotransferase and aspartate aminotransferase between the treated and control animals, but no statistically significant differences were obtained.

PERCENT OF CONTROL

	MALES				FEMALES				MALES				FEMALES			
eek	26	52	78	104	26	52	78	104	26	52	78	104	26	52	78	104
	ALANINE AMINOTRANSFERASE								ASPARTATE AMINOTRANSFERASE							
ow	71	86	96	102	84	112	103	94	81	70	92	132	74	190	100	114
id	86	79	120	88	83	98	98	75	97	60	120	91	69	100	100	90
igh	76	104	145	116	81	85	98	91	56	118	184	114	59	90	122	107

It is to be noted that, because of the wide variation in the clinical values obtained among the animals in the various groups, very few (2) statistically significant differences were obtained, although differences of up to 90% greater than control value and 53% below control value were reported between the treated and control animals.

4. Sacrifice and Pathology -

All animals that died or were sacrificed in extremis during the study were necropsied and gross observations were recorded. After 52 weeks of treatment, 10/sex/group and all survivors at week 104 were sacrificed and subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	X	Brain*†
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	X	Adrenals*
X	Cecum*	X	Kidneys*†	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*#
X	Rectum*	X	Testes*†	X	Parathyroids*
X	Liver*†	X	Epididymides	X	Thyroids*
	Gall bladder*	X	Prostate		Other
X	Pancreas*	X	Seminal vesicle	X	Bone*
	Respiratory	X	Ovaries*†	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin*
X	Lung*	X	Cervix/vagina	X	All gross lesions and masses*
X	Nose			X	Head
X	Pharynx				
X	Larynx				

* Required for chronic studies
\# In females only
† Organ weights required in chronic studies

a. Organ weight

The following organs were weighed (for the 10/sex/group sacrificed at 53 weeks and all survivors) at necropsy: brain (including stem), kidneys, liver, spleen, thyroid/parathyroid, and testes/epididymides.

Results

Absolute thyroid with parathyroid weight, thyroid with parathyroid/body weight ratios, and thyroid with parathyroid/brain weight ratios were significantly increased in the high-dose males at interim sacrifice.

	C	L	M	H
absolute	.044	.042	.046	.062*
thyroid/body weight	.0074	.0068	.0074	.0104*
thyroid/brain weight	.0194	.0184	.0202	.0275*

All other organ weights were comparable to control at interim sacrifice, although marginal differences (slight increase in liver weight was seen in the high-dose males and all treated females; slight decrease in kidney weight in the mid- and high-dose males) were reported.

Organ weight data at terminal sacrifice were reported as comparable among the groups.

b. Gross pathology

All animals on the study were subjected to detailed necrospy.

Results

Macroscopic findings of the animals dying during the study were reported as unremarkable. A slight increase in the incidence of enlarged pituitary glands and thickened mammary glands were observed in the high-dose females at interim sacrifice. At terminal sacrifice, a slight/marginal increase in the incidence of the following were noted, when compared to control.

HIGH-DOSE FEMALES

brain - indented ventral surface
pituitary - enlarged, irregularly shaped, mottled
liver - cyst, pale area
glandular stomach - filmy material on mucosa
mammary gland - thickened, fluid

HIGH-DOSE MALES

foot/paw - sore/swollen
lymph nodes - cyst, unequally sized

c. Microscopic pathology

All animals on the study were subjected to an extensive histological examination.

Results

The only histopathology findings reported were in the liver, pituitary, and the thyroids.

Slightly higher incidences of (a) clear cell cytoplasmic alteration in the high-dose females compared to control, and (b) centrilobular hepatocellular hypertrophy in both sexes at the high dose were reported

but were not considered to be biologically significant by the authors.

	MALES*				FEMALES*			
	C	L	M	H	C	L	M	H
cytoplasmic alteration								
basophilic	3	5	3	2	11	12	9	12
clear cell	1	2	3	7	2	0	0	1
eosinophilic	3	9	9	9	6	9	15	10
hepatocyte, hypertrophy,	1	0	0	1	0	1	1	1
centrilobular	2	0	0	9	3	0	4	11
vacuolation	8	3	4	5	9	4	4	6
single cell	7	9	9	9	12	18	15	11
hepatocellular carcinoma	0	2	2	2	0	0	0	0
hepatocellular adenoma	2	2	0	1	2	2	1	3

*N is 60 in all cases

The most common neoplasma, which was not discussed by the author, was pituitary adenoma/carcinoma.

		MALES				FEMALES			
PITUITARY		C	L	M	H	C	L	M	H
	N=	56	57	59	59	59	59	59	60
hyperplasia		9	10	8	11	5	2	3	4
B-adenoma		23	24	17	25	17	22	14	16
M-carcinoma		12	7	10	7	28	24	30	33
combined adenoma/ carcinoma		35	31	27	32	45	46	44	49
N-squamous cell carcinoma		0	0	0	1	0	0	0	0

It is to be noted that none of the animals displayed both an adenoma and a carcinoma; however, a few of the tumor-bearers displayed hyperplasia as well. The first pituitary carcinoma (PC) and pituitary adenoma (PA) in each group occurred as follows.

Group	Days on Study			
	MALES		FEMALES	
	PC	PA	PC	PA
Control	425	517	423	436
Low	561	501	460	514*
Mid	418	561	441	489
High	500	382*	431	530

* one high-dose male and two low-dose females had PA at the interim sacrifice (368-369 days)

No historical control data on the incidence of PA and PC observed at the performing laboratory were submitted. Published data on the incidence of these lesions in Sprague-Dawley rats (Charles River) show a wide variation. In one report (Ref. 1), PA incidence in males was 12/60 and in females, 25/105; adenocarcinoma incidence was listed as 16/60 males and 28/105 females. Another reference (2) gives an incidence

of PA as 26/250 males (study on caloric restriction and tumor risk in males); 12/179 males and 25/181 females (composite tabulation of control groups in studies of anticancer agents); 4/44 males and 1/44 females; 48/535 females (untreated control groups-11 different laboratories). Two studies showed a higher incidence - 35/57 males and 35/56 females (26 month study on saccharin - semisynthetic diet); 7/20 males and 12/20 females (untreated controls). A tabulation of control data from studies performed on colors (C.S. Lin, personal communication) shows similar incidences of both adenoma and carcinoma as is seen in the present study.

Although not discussed by the authors per se, there was an increased incidence of ovarian tumors ($p<0.05$, reported) in the high-dose group compared to controls (Fishers' Exact).

Ovary	Control	Low	Mid	High
Benign granulosa/ theca cell tumor	0	0	1	4
Malignant granulosa/ theca cell tumor	0	0	0	1
Sarcoma, undifferentiated	0	0	1	0

No historical control incidence data were provided. In the above-mentioned color-study compilation, the incidence reported for granulosa cell and theca cell tumors ranged from zero out of 70 to 3/59. The majority of the studies showed one or zero in the controls.

With regard to the thyroid, a slightly higher incidence of thyroid C-cell neoplasia was observed in the high-dose males compared to control. These were not attributed to the test compound by the authors. Although the authors stated that the incidence observed was within historical control values, no data were submitted to support this statement.

	MALES				FEMALES			
THYROID N=	C 60	L 60	M 60	H 58	C 60	L 60	M 59	H 60
"C" cell hyperplasia	9	3	1	6	4	2	4	6
B-"C" cell adenoma	1	1	1	4	1	0	0	3
M-"C" cell carcinoma	0	1	1	2	2	0	1	2
follicular cell hyperplasia	0	0	0	0	0	1	1	0
B-follicular cell adenoma	2	0	0	1	1	2	1	2
M-follicular cell carcinoma	3	3	0	0	1	1	0	0

None of the animals displayed more than one thyroid tumor, although a few did display hyperplasia in addition to the tumor. With the exception of one low-dose male ("C" cell adenoma at 684 days), all thyroid tumors in the males were found at the terminal sacrifice. In females, the thyroid tumors were seen at terminal sacrifice, with 4 exceptions: "C" cell adenoma - one control at 624 days and one high-dose at 713 days; follicular cell carcinoma - one control at 624

days and one low-dose at 705 days. The incidence of these tumors is similar to the incidence seen in several of the above-mentioned color studies.

D. DISCUSSION

There were no effects reported on body weight, food consumption, survival, clinical parameters, organ weights, or tumor incidence (with the exception of a slight increase in thyroid and ovarian tumors), suggesting an MTD was not tested. Additionally, it is noted that there were increases in several parameters in the liver (liver weight, clear cell alteration, centrilobular hepatocellular hypertrophy at the high dose (both sexes), and AST and ALT levels in the high-dose males), but none of these reached a statistical level of significance and the increases were not always dose-related. No discussion was provided by the authors as to how the dose levels were determined. In the 3-month mouse study (MRID # 256450), the dose of 500 ppm (HDT in chronic/onco study) was the NOEL, with decreases (statistically significant) in body weight reported at the mid- (1500 ppm) and high- (5000 ppm) dose levels. The decreases were usually within 10% of the control values, and decreases in food consumption were noted at various time intervals, suggesting a palatability problem. The liver weight increases and lesions observed were interpreted by the authors as indicative of adaptation. Therefore, it would appear that the 500 ppm (NOEL) dose level should not have been chosen as the highest dose to be tested in the chronic/onco study, but rather as the mid- or low-dose.

The RfD for this chemical (as of 4/8/86) is 0.013 mg/kg/day, based on a 1964 90-day feeding study in rats. The NOEL for that study was 13.3 mg/kg; the LOEL 133.3 mg/kg. The current study dose levels were (approximately) 0.6, 3.0, and 30 mg/kg.

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3-Month Mouse Feeding Study
(MRID # 256450)

% Control Food Consumption/% Control Body Weight at End of Week Interval

MALES	% Control		
week interval	Low	Mid	High
1-8	98.7/100	94.6/96.7	72.2/87.0
8-15	95.3/99.1	95.5/94.8	83.6/89.5
15-22	96.1/99.4	88.2/90.1	89.1/90.0
22-29	107.9/99.1	99.7/97.1	101.1/90.0
29-36	100.5/97.8	103.1/94.1	101.7/88.2
36-43	100.5/98.1	96.0/95.0	96.1/89.0
43-50	101.4/96.5	101.4/93.3	97.7/86.9
50-58	93.6/96.6	92.5/93.9	92.3/87.6
58-65	107.5/95.8	109.4/93.5	104.8/88.3
65-72	107.1/95.9	105.8/93.3	109.9/87.9
72-78	112.3/95.9	110.9/93.0	109.0/87.3
78-85	113.6/95.7	101.9/92.9	105.6/87.6
85-92	95.8/98.4	97.7/95.6	102.7/91.1

FEMALES	% Control		
week interval	Low	Mid	High
2-9	94.2/97.6	88.1/94.5	66.1/89.0
9-16	96.4/98.1	96.8/94.0	86.2/90.6
16-23	110.6/96.4	108.9/93.5	89.1/89.9
23-30	102.7/96.1	102.6/94.2	92.3/89.4
30-37	103.0/97.3	105.3/90.4	92.3/89.0
37-44	95.8/98.0	89.2/93.3	82.0/91.6
44-51	101.8/97.4	94.9/93.7	82.3/95.4
51-58	107.9/96.6	97.4/93.6	80.3/96.5
58-65	111.8/97.7	102.7/93.1	83.6/96.7
65-72	89.1/97.7	97.4/93.1	76.4/95.8
72-78	101.4/96.7	100.8/90.9	90.8/93.5
78-85	99.7/99.0	98.0/93.1	79.2/95.4
85-92	93.3/98.4	97.7/92.2	75.8/95.8

REFENENCES

1. Prejean, J.D., Peckham, J.C., Casey, A.E., Griswold, D.P., Weisburger, E.K., and Weisburger, J.H. Spontaneous Tumors in Sprague-Dawley Rats and Swiss Mice. Cancer Research 33, 2768-2773 (1973).

2. CRC Critical Reviews in Toxicology. March 1982.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/5/88
Tox. Branch II, Section II (TS-769C)
Secondary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/6/88
Acting Head, Section II, Tox. Branch II (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: oncogenicity study - mouse TOX. CHEM. NO.: 194

MRID NO.: 401625-01

TEST MATERIAL: Propachlor; 2-chloro-N-(1-methylethyl)-N-phenylacetamide

SYNONYMS: Ramrod®

STUDY NUMBER: HL-83-349; Project No. 241-159

SPONSOR: Monsanto Company

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Oncogenicity Study in Mice

AUTHOR: N.N. Hamada

REPORT ISSUED: January 19, 1987

CONCLUSIONS: Under the conditions of the study, Propachlor showed no oncogenic potential at dose levels up to 500 ppm. However, the dose levels used do not appear to have been high enough to adequately assess the oncogenic potential of Propachlor.

Classification: Core supplementary, pending submission of an acceptable rationale for terminating the study after 78 weeks when survival was excellent, and a discussion as to why a dose higher than the no-effect dose of the 90-day study was not utilized to insure an effective dose in the long-term study.

QUALITY ASSURANCE: A quality assurance statement was provided.

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A. MATERIALS:

1. Test Compound: Propachlor; Description: light brown flaky powder containing granular material of the same color; Batch #: Lot # MDRF 1114B; Purity: 96.1%:

2. Test Animals: Species: mouse, Strain: CD-1, Age: approximately 8 weeks old; Weight: males:26-36 grams; females:19-28 grams; Source: Charles River Breeding Laboratories, Inc., Portage, Michigan.

3. Statistics: Cumulative survival data through week 78 - National Cancer Institute Package (Thomas, et al., 1977); trend analysis of survival was evaluated at the 5% one-tailed probability level; statistical analyses were performed as diagrammed in Figure 1, attached.

   Absolute body weights, total food consumption, clinical pathology data (except leukocyte differentials and erythrocyte morphology), and organ weight data were examined (control vs treated per sex) as diagrammed in Figure 2, attached.

   Statistical analysis of neoplastic tumors was not performed due to the low incidence of tumors in all groups.

B. STUDY DESIGN:

1. Methodology: Mice (60/sex/group; housed individually) were administered Propachlor in the diet at dose levels of 0, 10, 50, or 500 ppm for 78 weeks. The dose levels were chosen (apparently) based on the 3-month oral dose study. Feed (Purina Certified Rodent Chow® #5002) and water were provided ad libitum. No vehicle was used. At 52 weeks, 10 animals/ sex/group were sacrificed and subjected to necropsy. All survivors were sacrificed at 78 weeks.

   Results:

   Compound Intake

   Problems were identified prior to study initiation with regard to the amount of Propachlor in the diets. It appeared that moderate losses of Propachlor occurred upon storage. Two separate studies (EHL 85046 and EHL 87105; MRID # 404731-02) were performed to investigate the fate of Propachlor in aged rodent diet. The first suggested that the apparent decline observed in both this 3-month study and in the chronic rodent study was a result of decreased extractability rather than degradation or volatility. The second study demonstrated that the levels found were indeed due to decreased extractability (increased binding to the diet) and not to degradation or volatility. It was concluded that a better recovery would have been obtained if the analysis in this study had been performed with a different solvent and more vigorous extraction procedures employed. At a maximum, 10% of the Propachlor could be lost if degradation occurred on storage of the treated diet. Therefore, the levels tested were probably close to the intended levels.

	Propachlor Consumed* (mg/kg)	
	Males	Females
Low dose	1.62	2.01
Mid dose	8.12	10.03
High dose	81.25	104.89

* values reported in final report, based on initial dietary concentration

2. Clinical Observations: The animals were observed twice daily for mortality and morbidity, with a full examination being performed twice weekly for signs of toxicity or ill health and for the presence of lesions or palpable tissue masses. Individual body weights were recorded at study initiation, and both body weight and food consumption were recorded at weekly intervals for weeks 1-14 and monthly thereafter.

Results: There were no differences reported in male survival throughout the study among the groups. Lower survival (statistically significant) was reported for all of the female dose groups, but there was no linear trend. These differences were not considered by the author to be compound-related due to the unusually high survival rate of the control females (90%). Historical control survival data suggests a 73% survival rate.

	Adjusted Survival (%)	
	Males	Females
Control	76	88
Low dose	74	62
Mid dose	78	70
High dose	67	67

The most common cause of death was listed as "not determined".

	Males	Females
Control	7/12	6/6
Low	4/13	10/19
Mid	7/12	13/15
High	11/16	12/16

Body weight and food consumption were said to be comparable among the groups for both sexes throughout the study. Transient differences in body weight changes/values/growth rates were not considered as related to treatment. A statistically significant increase in body-weight change was reported in the low-dose males through week 50, while a statistically significant decrease was reported in the mid-dose females at this time period. The mid-dose females also showed a statistically significant decrease in mean body-weight values and lower growth rates compared to control females through week 50. There were no differences reported in any of the other parameters monitored during the study. The changes observed were said to be those common to this mouse strain. Comment: In reviewing the Summary Table of Clinical Signs, this reviewer noted that the number of times the observations - Hunched and Thin

were reported was increased in both sexes in the mid- and high-dose groups.

	HUNCHED		THIN	
	Males	Females	Males	Females
Control	8	17	21	24
Low	8	19	22	29
Mid	24	32	34	44
High	29	30	38	40

3. Hematology: Blood samples were obtained from 10 animals/sex/group following 52 and 78 weeks of treatment. The CHECKED (X) parameters were examined.

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)		Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)		Mean corpuscular volume (MCV)
X	Platelet count		Reticulocyte count

Results: A statistically significant increase in segmented leukocytes was observed in the high-dose females at 52 weeks. This increase was not observed at termination and was not considered to be related to treatment. No differences were noted in any of the other parameters monitored.

5. Gross Pathology: All animals that died or were sacrificed in extremis were necropsied and gross observations were recorded. The animals sacrificed at 52 and 78 weeks were subjected to a macroscopic examination of:

the external surface
all orifices
cranial cavity
carcass
external surface of brain and spinal cord
nasal cavity and paranasal sinuses
thoracic, abdominal, and pelvic cavities and their viscera
cervical tissues and organs

The following organs were weighed and organ-to-body weight and organ-to-brain weight ratios were determined.

brain (including brain stem)
liver with gall bladder
kidneys
testes with epididymides
spleen
thyroid with parathyroid

Results: No gross pathological changes differences were observed among the groups at necropsy that could be related to treatment. Liver (with gallbladder)-to-body weight ratio was increased (15-16%) in the mid- and high-dose females, and the kidney-to-body weight ratio was decreased (12%) in the high-dose males. There was no pathology associated with these weight differences, but they are consistent with the changes observed in the 90-day mouse study.

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6. Histology: The following CHECKED (X) organs/tissues were collected from all animals.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta	X	Brain (3 levels)
X	Salivary glands	X	Heart	X	Periph. nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes*	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	X	Adrenals
X	Cecum	X	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
X	Gall bladder	X	Prostate		Other
X	Pancreas	X	Seminal vesicle	X	Bone (femur)
	Respiratory	X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Cervix	X	All gross lesions and masses
X	Nose			X	Head
	Pharynx				Harderian gland
	Larynx				

* mandibular and mesenteric

7. Histopathology: The lesions observed at termination were comparable among the groups, and there was no evidence of any compound-related effect.

D. Discussion

There was good survival in all of the groups/both sexes (greater than 60%) at study termination at 78 weeks. No effects were noted on body weight or food consumption that could be related to treatment. No differences were reported among the groups with respect to hematology, gross pathology, and histopathology. No evidence of oncogenicity was seen under the conditions of this study.

It was noted by this reviewer that the incidence of a "hunched" and "thin" appearance was more frequent in the mid- and high-dose females and the high-dose males than in the other groups. There was an increase in the liver (with gallbladder)to-body weight ratio in the mid- and high-dose females and a decrease in the kidney-to-body weight ratio in the high-dose males at termination. Because there was excellent survival of all of the groups at 78 weeks, it is not evident why the study was terminated. It was stated in the final report that the dose levels were chosen based on the three-month mouse study (reviewed elsewhere). The NOEL was 500 ppm (according to the author), and this was predicted to be the MTD for chronic exposure. Although differences were noted in several parameters between treated and control animals in this 78-week study, it would appear that a higher dose could have been tolerated.

E. CONCLUSION: The Registrant should to requested to provide the rationale for terminating the study after 78 weeks, when survival in all groups was excellent. Additionally, the Registrant should provide a discussion of why the highest dose tested was the no-effect dose from the 90-day study and not a dose slightly higher to ensure an effective dose.

Based on the conditions of the study, Propachlor did not show oncogenic potential at dose levels up to 500 ppm. However, the dose levels utilized do not appear to have been high enough to adequately assess the oncogenic potential of Propachlor. Before a final determination can be made of the adequacy of this study for predicting oncogenic potential of Propachlor, the above information is required.

241-159

Figure 1

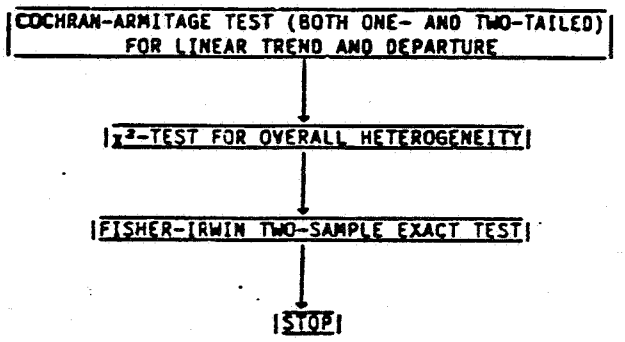

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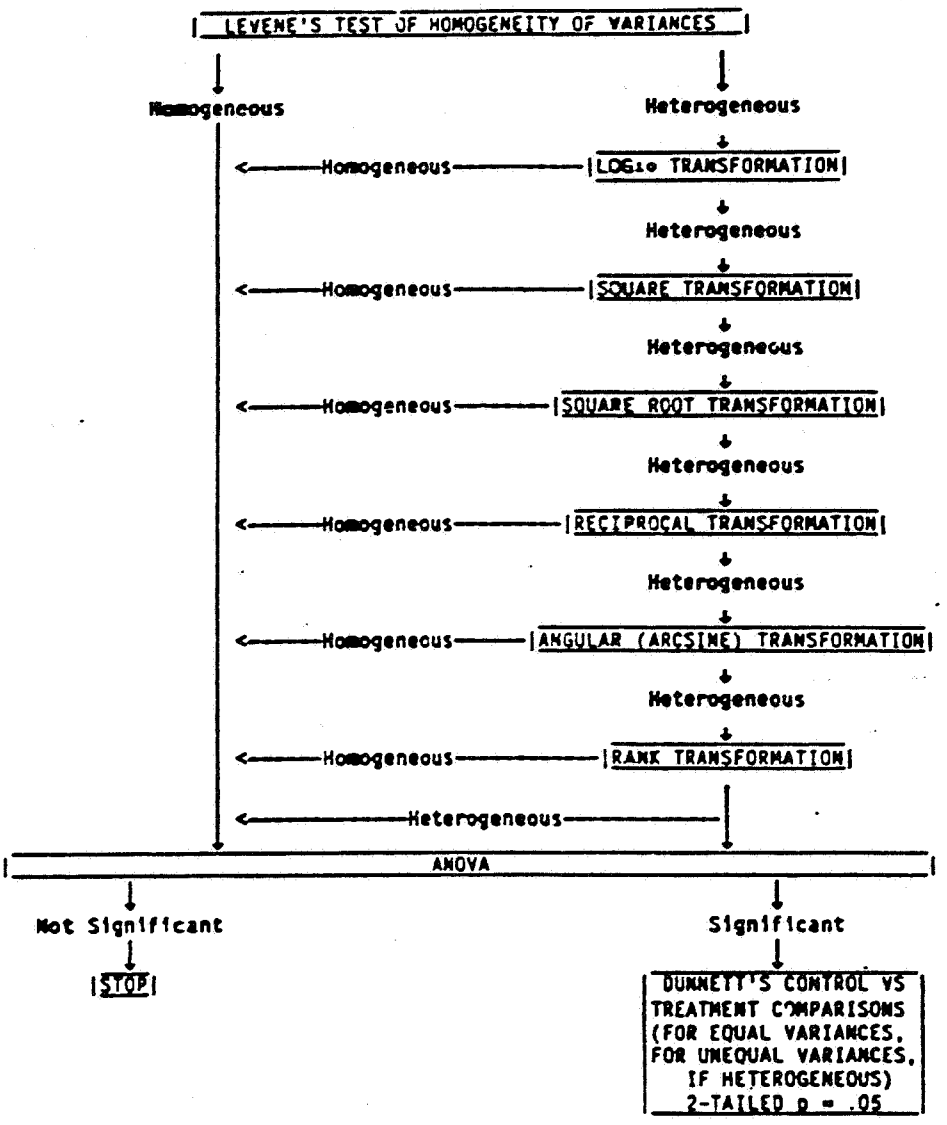
Figure 2



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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 12/5/88
Tox. Branch II, Section II (TS-769C)
Secondary Reviewer: James N. Rowe, Ph.D. James N. Rowe 12/6/88
Tox. Branch II, Acting Head Section II (TS-769C)
Date: October 24, 1988

DATA EVALUATION REPORT

STUDY TYPE: Three-Month Feeding - Mouse TOX. CHEM. NO.: 194

MRID NO.: 256450

TEST MATERIAL: 2-chloro-N-(1-methylethyl)-N-phenylacetamide

SYNONYMS: Propachlor, Ramrod®

STUDY NUMBER: 820061; Project No. ML-81-72

SPONSOR: Monsanto Company

TESTING FACILITY: Monsanto Environmental Health Laboratory

TITLE OF REPORT: Three Month Feeding Study Of Propachlor To Albino Mice

AUTHOR: M.S. Reyna and W.E. Ribelin

REPORT ISSUED: October 9, 1984

QUALITY ASSURANCE: A quality assurance statement was provided, although the study was performed prior to publication of the EPA's GLP regulations. A data audit was performed on this study during December 9-11, 1986 and several deficiencies were identified (see memo attached, dated July 8, 1987).

CONCLUSIONS: There was a decrease ($\leq$ 12% of control) in body weight observed in the mid- and high-dose animals, due primarily to a palatability problem, although in the males it may be due also to compound toxicity. Increased liver weight was reported in both sexes (mid-dose males and high dose both), which was accompanied by hepatocyte (centrilobular) hypertrophy (mainly in the males). The data audit suggested that the liver effects occurred at all dose levels (500, 1500, and 5000 ppm). Other effects noted include a dose-related decrease in absolute kidney weight in males, an increased relative brain weight (mid- and high-dose males), and a dose-related decrease in leukocytes in both sexes at week 7, which persisted to termination only in the high-dose males. The liver effects were attributed (by the authors) to an adaptation of the animals to the compound insult. The NOEL cannot be set at this time.

Classification: Core supplementary. Questions raised in a data audit of this study have not been addressed by the authors (as far as this reviewer can determine). No NOEL can be established for Propachlor exposure for 3 months in the mouse. Since there is an oncogenicity study available (MRID # 40162501) in the mouse, long-term effects on the liver can be assessed using that study, and a repeat of the 3-month mouse study is not necessary at this time.

A. MATERIALS:

1. Test Compound: Propachlor; Description: white crystalline solid; Batch (Lot) #: MTRF 1106B; Purity: 96.1%, according to the sponsor.

2. Test Animals: Species: mouse; Strain: Crl: CD®-1 (ICR) BR outbred albino; Age: approximately 5 weeks old at study initiation; Weight: males 24-33 grams, females 20-29 grams; Source: Charles River Breeding Laboratories, Portage, MI; Lot No. L82036.

3. Statistics: Body weight, food consumption, terminal body weight, and absolute organ weight - analysis of variance and Dunnett's test (two-tailed); hematology data - Dunnett's test for the comparison of multiple treatments with a control and/or by inspection; relative organ weights - Mann-Whitney test using the Bonferoni Inequality procedure for the comparison of unpaired samples; significance of differences between summary incidences of microscopic lesions in control and treated groups - Fisher Exact Test with the Bonferoni Inequality procedure for comparing unequal groups.

B. STUDY DESIGN:

1. Methodology: Mice (30/sex/group; housed individually, randomly assigned) were administered Propachlor (dissolved in acetone) in the diet at dose levels of 0, 500, 1500, and 5000 ppm for 3 months. Feed and water were provided ad libitum. Diets were prepared on a weekly basis.

Results

Compound Intake

Problems were identified early in the study with regard to the amount of Propachlor in the diets. It appeared that moderate losses of Propachlor occurred upon storage. Levels of Propachlor in the diets during the study were reported as follows:

Test Group	Target Level (ppm)	Analytical Level (ppm)[a]
Low	500	385 ± 35
Mid	1,500	1121 ± 86
High	5,000	3861 ± 166

[a] Average of determinations at days 7 and 14 (after one week's refrigeration and then following one week in the animal room)

Note: Two separate studies (EHL 85046 and EHL 87105; MRID # 404731-02) were performed to investigate the fate of Propachlor in aged rodent diet. The first suggested that the apparent decline observed in both this 3-month study and in the chronic rodent study was a result of decreased extractability rather than degradation or volatility. The second study demonstrated that the levels found were indeed due to decreased extractability (increased binding to the diet) and not to degradation or volatility. It was concluded that a better recovery would have been obtained if the analysis in this study had been performed with a different

solvent and more vigorous extraction procedures employed. At a maximum, 10% of the Propachlor could be lost if degradation occurred on storage of the treated diet. Therefore, the levels tested were probably close to the intended levels.

2. Clinical Observations: The animals were observed twice daily for mortality. Individual body weight, food consumption, and signs of toxicity were determined on a weekly basis. Note: The data audit raised issue with the quality of the clinical observations (see forementioned memo, page 2).

Results

Survival and Clinical Observations

All mice survived to study termination. No unusual observations were reported that might have been related to compound exposure.

Body Weight and Food Consumption

There was a decrease in body weight at all dose levels in both sexes at various time points during the study, as well as a decrease in food consumption, as shown below. The table shows for each group the percentage of control value for food consumption/% of control value for body weight (measured at the end of the week interval used for food consumption). High-dose males displayed an initial decrease in food consumption, but the decreased body weight (86.9-91.1% of control) persisted throughout the study. High-dose females displayed a consistent decrease in food consumption throughout the study, although the decrease (89.0-96.5% of control) in body weight was less than that observed in the high-dose males.

MALES % Control (food consumption/body weight)

week interval	Low	Mid	High
1-8	98.7/100	94.6/96.7	72.2/87.0
8-15	95.3/99.1	95.5/94.8	83.6/89.5
15-22	96.1/99.4	88.2/90.1	89.1/90.0
22-29	107.9/99.1	99.7/97.1	101.1/90.0
29-36	100.5/97.8	103.1/94.1	101.7/88.2
36-43	100.5/98.1	96.0/95.0	96.1/89.0
43-50	101.4/96.5	101.4/93.3	97.7/86.9
50-58	93.6/96.6	92.5/93.9	92.3/87.6
58-65	107.5/95.8	109.4/93.5	104.8/88.3
65-72	107.1/95.9	105.8/93.3	109.9/87.9
72-78	112.3/95.9	110.9/93.0	109.0/87.3
78-85	113.6/95.7	101.9/92.9	105.6/87.6
85-92	95.8/98.4	97.7/95.6	102.7/91.1

FEMALES — % Control (food consumption/body weight)

week interval	Low	Mid	High
2-9	94.2/97.6	88.1/94.5	66.1/89.0
9-16	96.4/98.1	96.8/94.0	86.2/90.6
16-23	110.6/96.4	108.9/93.5	89.1/89.9
23-30	102.7/96.1	102.6/94.2	92.3/89.4
30-37	103.0/97.3	105.3/90.4	92.3/89.0
37-44	95.8/98.0	89.2/93.3	82.0/91.6
44-51	101.8/97.4	94.9/93.7	82.3/95.4
51-58	107.9/96.6	97.4/93.6	80.3/96.5
58-65	111.8/97.7	102.7/93.1	83.6/96.7
65-72	89.1/97.7	97.4/93.1	76.4/95.8
72-78	101.4/96.7	100.8/90.9	90.8/93.5
78-85	99.7/99.0	98.0/93.1	79.2/95.4
85-92	93.3/98.4	97.7/92.2	75.8/95.8

3. Hematology: Blood samples were obtained from 10 animal/sex prior to study and at weeks 7 and 14 (10 animals/sex/group). The CHECKED (X) parameters were examined.

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB conc (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count	X	Reticulocyte count

Results

A dose-related decrease was observed in leukocytes (WBC) in both sexes at all dose levels at week 7; only the low-dose females did not attain a $p<0.05$. At termination, only the high-dose males displayed a statistically significant decrease in this parameter, although the mid-dose males values were also decreased. Other changes noted include:

Parameter (Week 7)	Dose/sex	Effect	Parameter (Week 14)	Dose/sex	Effect
Hgb	Mid/M	increased	MCHC	Mid/M	increased
Hct	Mid/M	increased	Retic	High/M	increased
RBC	Low/F	increased			

4. Gross Pathology: Twenty animals per sex per group were subjected to a full macroscopic examination of all tissues and organs in situ. The following organs were weighed: brain, heart, kidneys, liver, testes. The 10 animals/sex/group sacrificed at seven weeks for hematological examination were not necropsied. The final report stated that the animals were fasted overnight prior to sacrifice; the data audit determined that this is an incorrect statement.

Results

No treatment-related lesions were reported in any of the groups that could be attributed to compound exposure. There was a dose-related decrease in kidney weight in males that was statistically significant

at all dose levels. Additionally, liver weight was significantly increased in the mid- and high-dose males (dose-related) and in the high-dose females. Relative brain weight was significantly increased in the mid- and high-dose males; relative kidney weight (left and right, individually) was significantly decreased in the high-dose males; and a dose-related significant increase was reported in the relative liver weight at all dose levels in males and in the mid- and high-dose females.

5. Histology: The following organs/tissues were collected from all animals at terminal sacrifice.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart		Periph. nerve (sciatic)
X	Esophagus	X	Bone marrow		Spinal cord (3 levels)
X	Stomach	X	Lymph nodes*	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic n.)
	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	X	Adrenals
	Cecum	X	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
	Rectum	X	Testes	X	Parathyroids
X	Liver		Epididymides	X	Thyroids
	Gall bladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone (femur)
	Respiratory	X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung**		Cervix	X	All gross lesions and masses
	Nose				Head
	Pharynx				
	Larynx				

* mandibular and mesenteric
** with mainstem bronchi

Results

The main microscopic findings were in the liver, which consisted of increased hepatocyte enlargement (centrilobular) in 19/20 high-dose males, 8/20 mid-dose males, 2/20 low-dose males, and 3/20 high-dose females. Enlargement did not occur in any control livers. Another microscopic finding was the absence of the outer retinal plexiform and rod and cone layers of the eyes in 4 high-dose males, 1 control male, 2 high-dose females and 1 control female.

Comment: The data audit indicated that the livers of the low- and mid-dose males were examined microscopically, while the female livers were not (a protocol deviation). Additionally, a reanalysis of the dose-dependent incidence of centrolobular enlargement in the male liver (and the incidence in the females) suggested (to the auditors) that a NOEL was not attained. This was not discussed in the final report.

C. CONCLUSION:

Although the decrease in leukocytes was observed in all treated groups at 7 weeks and in the high-dose males at study termination, no explanation was put forth regarding this finding, which appeared to be real. Nor were microscopic abnormalities observed in any hematopoietic tissue to assist in interpretating this observation. In the chronic/oncogenicity study in rats, a comparable effect on leukocytes was not observed, although the first measurement time was not until 26 weeks. The effects in the current study appear to have dissipated by 14 weeks of treatment, except in the high-dose males.

Other effects observed were an increase in liver weight and increased hepatocyte (centrilobular) hypertrophy, which were attributed by the authors to a compensatory mechanism of the animal in response to compound insult. The males displayed the greater effect than the females. There were decreased kidney weights in males, but no corresponding microscopic changes. The differences noted between the sexes suggest a possible difference in the metabolism of this compound.

Because of the questions raised in the data audit of this study with regard to the liver effects, which apparently have not been addressed by the authors, a NOEL for this study cannot be set. However, since there is an oncogenicity study available on the mouse (Study No. HL-83-349/241-159; dated February 20, 1987; MRID # 40162501), there is no need for this three-month study to be repeated (at this time).

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

Rec'd Bill Burnam 11/17/87

JUL - 8 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: GLP Inspection/Study Audit Report; Request for Regulatory Review

FROM: Dexter S. Goldman, Director dsg
Laboratory Data Integrity Assurance Division (EN-342)

TO: Allen Jennings, Director
Benefits and Use Division (TS-768C)

Attached please find the report of the data audits conducted at Monsanto Company, Environmental Health Laboratory, St. Louis, MO, during the period of December 9 - 11, 1986.

The following studies were audited:

Chemical	Title	Study No.
Propachlor	Three-month Feeding Study to Albino Mice	EHL 820061
Propachlor	Three-month Feeding Study to Albino Rats	EHL 830037
Glyphosate	Twelve-month Oral Study to Beagle Dogs	EHL 830116
Acetochlor	Review of Test Substance Chemistry Studies (Dog, Rat, Mouse) Conducted by Tegeris Laboratories, Laurel, MD	

These studies were conducted prior to the publication of the EPA's GLP regulations, and submitted to the Agency.

The inspector's report is enclosed, and some of the major findings are highlighted in the following paragraphs.

While a comprehensive Good Laboratory Practice inspection was not conducted during this visit, several GLP-related issues were reviewed during the conduct of the audit. Some were specifically related to the audit, and one was a continuation of an earlier inspection related to the archives. The temporary archives at EHL are satisfactory and in compliance with the regulations. The main archives at the Warson Road facility are in full compliance, but they are located in a warehouse which might compromise the fire security of the otherwise well designed and controlled facility.

During the study audits, it was found that certain raw data records had not been retained and others had not been generated. In general, these dealt with chemistry aspects of the studies and are described in the individual audit reports. Most important in terms of regulatory review was the observation that dose analyses showed that up to 30% of the test chemical (propachlor) could not be recovered from the dosed food. This issue should have been resolved before proceeding with testing. This finding may also have a significant bearing an other long term feeding studies conducted with propachlor at other laboratories (e.s. Tegeris) for Monsanto.

Finally, statistical reanalysis of some parameters--detailed in the individual reports--suggests that additional data analyses should have been done to give a more complete description of the effects of the test chemical.

With certain exceptions, as detailed below and in the reports, the data audits tended to confirm the final reports. The significance of these exceptions should be considered in your review.

A. Propachlor mouse study:

1. Dose analyses in feed indicated that recovery of propachlor was low, and data at the laboratory was inconclusive to explain the cause. The audit team raised the question of the nature of the actual toxic agent tested.

2. Raw data relating to the test chemical purity assays and chemical stability were discarded contrary to the requirements for data retention.

3. Questions were raised about the quality of the animal room observations in that the singly caged mice were supposedly observed twice daily, yet one mouse was reported missing for three days (six observations) but was then found in the next cage on the following day.

4. There was inadequate documentation of tissue recuts.

5. The statement in the final report on fasting the animals prior to necropsy was incorrect.

6. The statistically significant dose-dependent decrease in kidney weights was discussed in the results and discussion sections of the final report but was not mentioned in either the summary or conclusions.

7. The final report was unclear as to the numbers of animals used for the test and whether or not the extra animals were used for bleedings; the record of selection of the animals for bleedings was not available.

8. The livers of the low- and mid-dose male animals were examined microscopically; the livers of the female animals were not. This is a protocol deviation. This is also despite the recorded dose-dependent increase in liver weights in the females.

9. Statistical reanalysis of the dose-dependent incidence of male animal centolobular hepatocyte enlargement suggests that this study did not determine the NOEL. A similar reanalysis of the female mouse liver weights again showed that there is no demonstrable NOEL. These were not adequately reported in the final report.

B. Propachlor rat study:

1. The same problems in test chemical characterization, assay, stability data retention, and poor recovery of test substance (propachlor) from feed, as noted in the mouse study, also apply to the rat study.

2. The absence of unscheduled deaths in the male high-dose group as well as the relative but not absolute weight gains in the high-dose group suggests that weight might not be a suitable guide for predicting the maximum tolerated (or effective) dose.

3. For necropsy and histology, the same comments apply relative to tissue recuts and selection of animals for bleeding.

4. Animals were exsanguinated under cold water. Saline exsanguination is to be preferred.

5. Statistical reanalysis of female liver weights suggests that there is a significant dose effect based on absolute and relative weights. The significance of this is that no NOEL was demonstrated in this study; the final report did not include this result.

C. Glyphosate (12-Month Dog) Study:

1. Statistical reanalysis of the weights of the pituitaries of the male animals suggests that there was merit to the reviewer's concerns on these data. Registrant rounded off improperly the % relative weight, and the associated means were not useful in statistical evaluations.

2. Statistical reanalysis of clinical pathology values shows that there are dose-related trends which were not discussed in the report.

3. Raw data were missing for the stability of the test chemical over the study period.

D. In the special audit of the Acetochlor chemistry data (part of the health effects studies conducted at Tegeris), chromatograms for the assay raw data were missing, and there is no record that the test substance stability was determined either by sponsor or performing lab.

This report was submitted to the laboratory and their response is attached as an addendum to the report.

Please provide me, within 90 days, with a regulatory review of this report.

Along with the report I have enclosed background material and exhibits resulting from this inspection. This material is for your archives, but should be available to us if there is further need.

Attachment

cc: A. E. Conroy II (Memo only)

Douglas Campt (Memo only)

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Guideline Series 84 : MUTAGENICITY

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 10/26/88
Tox. Branch II, Section II (TS-768C)
Secondary reviewers: Marcia van Gemert, Ph.D.
Tox. Branch II, Acting Head Section II (TS-769C)
Kerry Dearfield, Ph.D.
Health Effects Division (TS-769C) Kerry Dearfield 10.28.88
Date: October 26, 1988

DATA EVALUATION REPORT

CHEMICAL: Propachlor Tox. Chem. No.: 194

STUDY TYPE: CHO/HGPRT Gene Mutation Assay with Propachlor

MRID NUMBER: 260238

SYNONYMS/CAS No.: Ramrod®; 2-chloro-N-(1-methylethyl)-N-phenylacetamide/ [1919-16-7]

SPONSOR: Monsanto Company

TESTING FACILITY: Monsanto Environmental Health Laboratory

TITLE OF REPORT: CHO/HGPRT Gene Mutation Assay with Propachlor

AUTHOR(S): Leonard J. Flowers

STUDY NUMBER(S): ML-84-237; EHL # 840083

REPORT ISSUED: August 2, 1985

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION(S) - Executive Summary: Under the conditions of this assay, Propachlor induced a concentration-dependent increase over the range 30-50 ug/ml to over a doubling of the solvent control mutant frequency at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus of Chinese hamster ovary cells, with metabolic activation. No apparent increase was obtained without activation.

Classification: Acceptable. In light of the positive activity, it will be assumed that Propachlor is positive in this assay despite the lack of documentation detailing the nature and grade of Propachlor used. The Registrant should provide such documentation.

I. Materials and Methods: Propachlor (CP 31393, Lot # MDRF 1114B) was described as a yellow-tan solid with a purity of 96.1%. The subclone K_1BH_4 of Chinese hamster ovary cells (CHO), originally obtained from Dr. A.W. Hsie of Oak Ridge National Laboratories, were used; these were routinely maintained as log growing monolayer cultures in Ham's F12 medium, supplemented with heat-inactivated 10% fetal calf serum. There was one negative control: solvent ethanol. The positive control agents were benzo(a)pyrene [B(a)P] and ethyl methane sulfonate (EMS) for both the activation and non-activation assays, respectively.

The assays were conducted with and without metabolic activation. The activated systems utilized the S9 fraction derived from the liver of rats induced with Aroclor 1254. The S9 cofactor mixture contained 50 mM sodium phosphate (pH 7.5), 4 mM NADP, 0.5 mM glucose-6-phosphate, 30 mM KCl, 10 mM $CaCl_2$, and different amounts of liver S9. The different concentrations of S9 were said to represent the % of S9 (v/v) in the S9/cofactor mixtures (1 ml of the S9/cofactor mixture was added to 4 ml of medium for cytotoxicity or mutagenicity testing.

Cytotoxicity Determination - CHO cells were seeded at 0.5 X 10^6 cells per flask in growth medium 18-24 hours prior to treatment. The medium was changed to Ham's F12 medium without serum with and without activation on the day of treatment. Test material was added at various concentrations and, after 3 hours' incubation, the treated medium was discarded. Approximately 200 cells were plated per sample for the determination of cloning efficiency (incubation 7-9 days). Cytotoxicity was expressed as relative survival.

$$\text{Cloning efficiency (CE)} = \frac{\text{no. of colonies}}{\text{no. of cells plated}}$$

$$\text{Relative survival (RS)} = \frac{\text{CE (treated)}}{\text{CE (control)}}$$

Mutagenesis - CHO cells were plated one day before treatment. On the day of treatment, cells were treated with test material, positive controls, and negative solvent control. The cells were then processed as described above for cytotoxicity determination plus 10^6 cells per sample were plated in subculture medium (hypoxanthine-free Ham's F12 medium supplemented with 10% dialyzed newborn calf serum). The cells were subcultured every 2-3 days as unattached cultures for 7-9 days for the expression of phenotype. Mutant selection was performed using selective medium (hypoxanthine-free Ham's F12 medium supplemented with 10 uM 6-thioguanine (6-TG) and 5% dialyzed newborn calf serum). Three aliquots of approximately 200 cells per sample were plated in selective medium without 6-TG for the determination of cloning efficiency. The colonies developed were fixed, stained, and counted. The results were expressed as mutant frequency (MF).

$$MF = \frac{\text{no. of mutant colonies}}{\text{no. of cells plated}} \times \frac{1}{CE}$$

Range-finding Studies - Range-finding studies were performed to determine the cytotoxicity of the test material, using a wide range of concentrations of test material and S-9. Additionally, the dose response in mutagenicity was tested at varying S-9 concentrations.

Statistical Analysis - Statistical analysis was based on the methods of Snee and Irr (Mutation Res. 85, 77-93 (1981)), which consist of transformation of the data followed by an analysis for a linear, quadratic, or higher-order dose-response relationship and pairwise comparison of control and treated data.

II. Results:

A. Solubility, Stability, and Dose Determination - Solutions of the test material were made on the day of treatment using ethanol as the solvent. No details of the composition of the test material were provided other than purity. It was stated that no stability problems were reported; however, in the chronic/oncogenicity study, it was stated that the test material was stable up to 3 months under freezer conditions. In the present study, the test material was stored at room temperature, was received on 1/13/84, and the study was initiated on 8/21/84 and completed on 2/21/85.

The doses selected for testing the initial mutagenic potential of Propachlor were 10, 40, and 50 ug/ml, using S-9 at 0, 2, 5, and 10%. A subsequent study using Propachlor at 10, 30, 40, 50, and 60 ug/ml was also performed with (5%) and without (0%) S-9.

B. Cytotoxicity - Substantial cytotoxicity (>50% cell killing) was observed at doses of 40 ug/ml and higher in the range-finding study (see Appendix I, Tables 1 & 2, attached). Colony counts were comparable to control up to 30 ug/ml at the S-9 dose levels tested, although the counts at 30 ug/ml without S-9 and at 1% S-9 were slightly lower than control. In the second range-finding study, colony counts of 20 ug/ml and below were comparable to control at the various S-9 concentrations, with 5% and 10% being optimal.

C. Mutagenic Potential - No statistically significant increases in mutant frequency were observed in the preliminary studies at dose levels of 10, 40, and 50 ug/ml Propachlor with (1, 2, 5, & 10%) and without S-9 (see attached Appendix I, Table 3). Propachlor was tested at doses of 10, 30, 40, 50, and 60 ug/ml, with and without S-9 (5%). The authors stated that no statistically significant increases were found in mutant frequency in the Propachlor-treated cultures (see Appendix I, Table 4, attached). Note: The positive controls were said to have yielded the expected positive responses in mutagenicity (see Appendix I, Table 5, attached).

III. Conclusion: While there does not appear to be a significant increase in mutant frequency over ethanol control values without activation, there is a concentration-dependent increase in mutant frequency (30-50 ug/ml) to over a doubling of ethanol control frequency at 50 ug/ml with 5% S9 present (Appendix I, Table 4, attached). Although all mutation frequencies are within the usual range of 0-20 x 10^{-6} for spontaneous background, this laboratory, from the data presented in many initial experiments and the final experiment (Appendix I, Tables 3 and 4), reports a good background range of 0.7-7.8 x 10^{-6} for the ethanol control with the presence of S9. The raw colony counts (appendix II, Table 4, attached) also indicate an absolute elevation of colonies in selection medium at 50 ug/ml. Therefore, based on the concentration dependent increase, doubling of background, appropriate toxicity (14% relative survival at 50 ug/ml), increase in absolute colony numbers, and the relatively tight spontaneous background, it appears that the test compound is weakly mutagenic in this assay under activated conditions.

NOTE: Although the report states that no stability problems were identified, it is to be noted that the chronic/oncogenicity study (rat) (MRID # 404731-01) study final report stated that Propachlor is stable up to 3 months under freezer conditions; the Propachlor used in this assay was stored at room temperature and, apparently, for a considerable time (received on January 13, 1984; study was initiated on August 21, 1984 and completed on February 21, 1985). In the rat bone marrow assay report, a Note To Reviewer stated that Technical Propachlor (96.3%) showed negligible change (0.2% decrease) in that assay after storage at 86° C (187°F) for 16 days in contact with a 304 stainless steel coupon to simulate Propachlor tank storage. Additionally, this note stated that other studies have demonstrated that Propachlor is stable (shows negligible decomposition) upon storage at room temperature (70-80°F) for over a year. In light of the positive activity, it will assumed that Propachlor is positive in this assay despite the lack of documentation detailing the nature and grade of Propachlor used.

Comment: An analogue, acetochlor, is reported in a Peer Review document to be weakly positive in this assay also.

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NOTE TO REVIEWER

The general acceptability of previously submitted in vivo cytogenetics tests sponsored by Monsanto has been questioned by Agency reviewers. The issue has been whether these studies have adequately demonstrated that the test material has reached the bone marrow. This issue was discussed with Dr. Irv Mauer in connection with a recently completed in vivo cytogenetics assay with propachlor, submitted herewith. An October 3, 1985 memo summarizing this discussion between Drs. A. P. Li and I. Mauer is included with this submission package.

The stability of technical propachlor has been demonstrated and reported to the EPA in a report "Product Chemistry Data to Support the Continued Registration of Propachlor" RD No. 630, Special Report MSL-4890, submission date July 30, 1985.

Technical Propachlor (96.3%) showed a negligible change (0.2% decrease) in assay after storage at 86°C (187°F) for 16 days in contact with a 304 stainless steel coupon to simulate propachlor tank storage.

Other studies have demonstrated that propachlor is stable (shows negligible decomposition) upon storage at room temperature 70-80°F for over the year.

Propachlor toxicology review

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___ Identity of product inert ingredients

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___ Description of the product manufacturing process

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___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

006972

Reviewed by: Linda L. Taylor, Ph.D. 9/26/88
Tox. Branch II, Section II (TS-769C)
Secondary reviewers: Marcia van Gemert, Ph.D.
Acting Head, Section II (TS-769C)
Kerry Dearfield, Ph.D.
Health Effects Division (TS-769C) 10.28.88
Date: October 26, 1988

DATA EVALUATION REPORT

CHEMICAL: Propachlor Tox. Chem. No.: 194

STUDY TYPE: Acute In Vivo Rat Bone Marrow Cytogenetics Assay

ACCESSION NUMBER: 260238

SYNONYMS/CAS No.: Ramrod®/1918-16-7

SPONSOR: Monsanto Company

TESTING FACILITY: SRI International

TITLE OF REPORT: An Assessment of the Mutagenic Potential of Propachlor Utilizing the Acute In Vivo Rat Bone Marrow Cytogenetics Assay

AUTHOR(S): Ted L. Ernst, M.S. and William F. Blazak, Ph.D.

STUDY NUMBER: SR-84-180; SRI Project No. LSC-7405

REPORT ISSUED: August, 1985

QUALITY ASSURANCE: A quality assurance statement was provided.

Classification: Unacceptable, pending submission of additional data to support the dose levels tested. Additionally, clarification of whether the test material used is the technical grade of Propachlor should be provided.

CONCLUSION(S) - Executive Summary: Under the conditions of this assay, Propachlor was not shown to be clastogenic to rat bone marrow cells following in vivo exposure at 0.05, 0.2, and 1.0 mg/kg given i.p. in ethanol (1 mg/kg). However, before a final determination can be made, the registrant should be requested to provide additional information to justify the low levels tested in this assay in light of other data (published literature), which indicate that Propachlor is positive for aberrations in mouse bone marrow and Alachlor (an analogue) is positive for aberrations in rat bone marrow.

[redacted]

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A. MATERIALS

1. Test Material: Name: 2-chloro-N-(1-methylethyl)-N-phenylacetamide; (Propachlor); Description: Light-brown crystalline flakes; Batch #: Lot MDRF 1015C; Purity: 95.8%; Solvent: absolute ethanol; Stability: see: Note To Reviewer (attached).

2. Control Materials: Negative: solvent control-absolute ethanol. Positive: Triethylenemelamine (TEM: CAS No. 51-18-3).

3. Test Organism: Bone marrow cells collected from the femurs of male and female adult Fischer-344 rats (Simonsen Laboratories, Inc., Gilroy, CA) were utilized in this assay.

4. Test Material Concentrations: The doses utilized in the definitive study were 0.05, 0.2, and 1.0 mg/kg, based on three pilot studies.

B. TEST PERFORMANCE

Pilot Studies - These studies were conducted to evaluate the effect of Propachlor on the mitotic index (MI, % metaphase cells) of bone marrow cells and on the health/survival of the animals. Animals were administered the test material (138, 225, 550, 1100, and 2200 mg/kg) or negative control in ethanol by intraperitoneal injection, and the survivors were sacrificed 24 hours or 14 days after treatment. Due to excessive mortality in the test material groups (61/70 dead within 24 hours) in the initial study, the 24-hour animals were pooled with the 14-day groups. Because few animals survived the 14-day observation pericd, a second 24-hour and 14-day pilot study were run with lower doses (12.5, 25, 50, and 200 mg/kg). After 24 hours, the designated animals were injected with colchicine (in HBSS) and were sacrificed 2-3 hours later. The number of metaphase cells observed in a sample of at least 1000 cells was scored, and an MI was calculated for each animal. Again, excessive mortality (46/50) occurred, and an LD_{50} could not be calculated. A third 14-day pilot study, with doses of 0.2, 1.0, and 5.0 mg/kg was performed. Animals were injected i.p. as above, observed for clinical signs of toxicity, and sacrificed after 14 days. An LD_{50} value was calculated using probit analysis.

Definitive Study - One hundred and thirty-two rats were treated as above with either test material of negative control article and the survivors were sacrificed at three different intervals; 6 hours, 12 hours, and 24 hours (groups consisted of 6/sex); positive controls (6/sex) were treated similarly, but only the 24-hour time period was used. Two to three hours prior to sacrifice, the animals were injected with colchinine in HSBB (4 mg/kg). The bone marrow cells were prepared according to a modification of the approach specified by Nichols, et al., (1972).

Slides of bone marrow cells were prepared, and slides from 5 animals

per treatment group per sex per sacrifice period were coded and analyzed separately ('blind") by three cytogeneticists. Slides were evaluated for mitotic index (based on at least 1000 cells/animal), and 60 cells per animal, when possible, were evaluated for chromosomal aberrations.

Score sheets were used to record pertinent information, including slide quality, MI, chromosome number, and numbers of various categories of chromatid and chromosomal aberrations for each cell scored (Savage, 1975). Chromatid and isochromatid gaps were recorded for each cell but were not considered chromosomal aberrations in the analysis data.

C. ANALYSIS OF DATA

1. Statistical Methods: VAX 11/782 computer programs were used. The statistics utilized are described on the attached sheet, page 12.

2. Criteria for a Valid Chromosomal Aberration Assay: (a) the chromosomal aberration frequencies in the positive control animals are significantly ($p<0.05$) elevated above those in the negative control animals and (b) chromosomal aberration frequency analyses are concordant among geneticists.

2. Criteria for Positive and Negative Results: If either the mean aberrant cell frequency or the mean chromosomal aberration frequency per cell, or both, was significantly greater ($p<0.05$) in the test material-treated animals than in the negative control animals, the compound is considered positive. A test material is considered negative if the results obtained by each cytogeneticist were in general agreement and the criteria for a positive response were not met. An inconclusive response would be one in which there were reason to believe that the concentrations of test material tested were inappropriate (excessive cytotoxicity or lack of any toxicity).

D. RESULTS

Clinical Observations: Eye and nose exudates, humped backs, and rough fur were the primary toxic signs reported, and these were said to be dose related. The mean body weight change in males, although not significantly different among the groups, was greater (more weight was lost) in the test material groups (but not dose-related) than in either the negative or positive control groups. For females, the weight change was least in the positive control (see Tables 13 and 14, attached).

Cytological Observations: Three measures of cytological damage were evaluated statistically - MI, the % of chromosomally aberrant cells, and the frequency of chromosomal aberrations per cell. No significant differences in any of the measures of cytological damage were reported between the negative control and the propachlor-

treated animals (see Tables 15-20, attached). The positive controls produced the expected response, thus demonstrating the ability of the assay to detect a clastogenic compound.

E. CONCLUSION

Although Propachlor was not shown to be clastogenic, under the conditions of this assay, to rat bone marrow cells following in vivo exposure, the dose levels tested are low and, without additional information, it cannot be determined whether the doses tested were appropriate.

With regard to the pilot studies, ethanol itself was shown to be lethal (2/2 deaths) to the females (apparently at a dose of 3.3 ml/kg). This dose was apparently lowered to 1.0 ml/kg in the other studies. In the latter pilot studies, no ethanol control animals were used. From the literature, the LDL_0 for ethanol in rats by the i.p. route is 1225 mg/kg. The third pilot study examined dose levels of 0.2, 1.0, and 5.0 mg/kg (no ethanol control), which resulted in some mortality (20-60%), but only one high-dose male died within 24 hours of dosing. It is not evident why the definitive study, which used sacrifice times up to 24 hours, utilized dose levels that are 5 times lower than these dose levels. Additionally, it is not evident that the effects observed are attributable entirely to Propachlor and not to the ethanol and/or the i.p. route of exposure. The Registrant should be requested to provide additional information to justify the low levels tested in this assay in light of other data (published literature), which indicate that Propachlor is positive for aberrations in mouse bone marrow (Pilinskaya, et al., 1980) and Alachlor (an analogue) (Georgian, et al.,1983) is positive for aberrations in rat bone marrow.

Additionally, the Registrant should provide clarification of whether the test material used is the technical grade of Propachlor.

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Nichols, W.W., Moorehead, P., and Brewen, G. Chromosome Methodologies in Mutation Testing; ad hoc committee report. Tox. and Appl. Pharm. 22, 269-275 (1972).

Savage, J.R.K. Classification and Relationships of Induced Chromosomal Structural Changes. J. Med. Genet. 12, 103-122 (1975).

Pilinskaya, M., Kurinyi, A., L'vovo, T., and German, I. Preliminary Evaluation of the Cytogenetic Activity and Potential Mutagenic Hazard of 22 Pesticides. Tsitol. Genet. 14, 41-47 (1980).

Georgian, L., Moraru, I., Draghicescu, T., Dinu, I., and Ghizelea, G. Cytogenetic Effects of Alachlor and Mancozeb. Mutation Research 116, 341-348 (1983).

NOTE TO REVIEWER

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Propachlor toxicology review

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~~006527~~

Guideline Series 84 : MUTAGENICITY

006972

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 10/26/88
Tox. Branch II, Section II (TS-768C)
Secondary reviewers: Marcia van Gemert, Ph.D.
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Date: October 26, 1988

DATA EVALUATION REPORT

CHEMICAL: Propachlor

Tox. Chem. No.: 194

EPA ID NO: 88-NY-03

STUDY TYPE: Chromosomal Aberration Frequencies - Induction in Chinese Hamster Ovary (CHO) Cells

ACCESSION NUMBER: 403127-01

SYNONYMS/CAS No.: Ramrod®/1918-16-7

SPONSOR: Monsanto Company

TESTING FACILITY: Monsanto Environmental Health Laboratory

TITLE OF REPORT: In Vitro Cytogenetic Study of Propachlor

AUTHOR(S): A.P. Li and C.A. Myers

STUDY NUMBER: MSL-6930 (Laboratory Project No. EHL-86047)

REPORT ISSUED: June 23, 1987

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION(S) - Executive Summary: Under the conditions of this assay, Propachlor was found to be negative for induction of chromosomal aberrations in Chinese hamster ovary cells without metabolic activation, at concentrations up to 15 ug/ml. There was a weak, but statistically significant, response at the 15 ug/ml dose level with metabolic activation at the 12- and 24-hour harvest times. Propachlor induced a clastogenic effect under metabolic activation conditions.

Classification: Acceptable. In light of the positive activity, it will be assumed that Propachlor is positive in this assay despite the lack of documentation detailing the nature and grade of Propachlor used. The Registrant should provide such documentation.

A. MATERIALS

1. Test Material: Name: 2-chloro-N-(1-methylethyl)-N-phenylacetamide; (Propachlor); Description: Brown flakes; Batch #: MIRF-01-05B; Purity: 97.4%; Solvent: ethanol; Solubility: limit - 3.08 mg/ml in ethanol.

2. Control Materials: Negative: ethanol (solvent control). Positive: methyl methane sulphonate (MMS in ethanol) for the non-activation series and cyclophosphamide (CP in Hank's balanced salt solution (HBSS)) in the metabolic activation series.

3. Test Organism: The Chinese hamster ovary (CHO) cells were routinely maintained in Ham's F12 medium, supplemented with 5% newborn calf serum.

4. Activation System: The assays were conducted with and without metabolic activation. The activated systems utilized commercially available (purchased from Litton Bionetics) Aroclor 1254-induced rat liver homogenate (S9) as the exogenous activation system. The S9/cofactor mixture contained 50 mM sodium phosphate (pH 7.5), 4 mM NADP, 0.5 mM glucose-6-phosphate, 30 mM KCL, 10 mM $MgCl_2$, 10 mM $CaCl_2$, and liver S9.

B. TEST PERFORMANCE

1. Range-finding: Approximately 500,000 CHO cells were seeded in medium and incubated for 18-24 hours prior to treatment. On the day of treatment, the medium was changed to medium with 10 uM 5-bromo-deoxyuridine (BrdU), used to label the chromosomes for average cell generation time (ACGT), and test material at eight concentrations ranging from 5 ug/ml to 3.08 mg/ml was added. A second range-finding study was performed using a narrower range of concentrations (5-20 ug/ml). The cells were returned to the incubator for 5 hours, after which the medium was changed to one containing only BrdU. Colchicine was added 2 hours before the cells were harvested 24 hours after treatment initiation. Approximately 500 cells per treatment were scored for mitotic index and 100 cells per treatment for ACGT. Mitotic index was determined by the ratio of metaphase cells to the total number of cells scored. ACGT was determined by the following equation:

$$1 \times \%\ 1^{st}\ \text{Division} + 2 \times \%\ 2^{nd}\ \text{Division} + 3 \times \%\ 3^{rd}\ \text{Division} = \frac{\text{hrs in BrdU}}{\text{ACGT}}$$

First division mitotic cells were identified by darkly stained chromatids; second division cells had differently stained sister chromatids (one light, one dark); third division cells had lightly stained chromatids.

2. Cytogenetics: Doses tested were chosen from the range-finding study. Duplicate samples per treatment condition were used. The dose levels of Propachlor were 1, 2, 5, 10, and 15 ug/ml with and without metabolic activation. The treatment and harvesting of cells were as described above except that BrdU was not present in the medium. The harvest times chosen were approximately 1/2, 1, and 2 X of the

ACGT for examination of cells treated at S/G2, G1/S, and possible effects that can persist to 2nd cell division, respectively. Where possible, 100 cells per sample (200 cells per treatment) were scored for both chromatid- and chromosome-type aberrations. The harvest times chosen were:

Concentration of Propachlor	Harvest Time
10 ug/ml and lower	6, 12, and 24 hours
15 ug/ml	6, 12, 24, and 36 hours

C. ANALYSIS OF DATA

Chi-square analysis and Dunnett's t-test were used to analyze the number of cells with structural aberrations and structural aberrations per cell, respectively. Dose-response was analyzed via linear regression analysis of response versus linear or log dose (Galloway, et al., 1985).

D. RESULTS

1. Range-finding Experiments - Cytotoxicity was observed at 20 ug/ml, as indicated by a lengthening of average cell generation time to approximately 18 hours in both the activated and non-activated assays. Concentrations above 20 ug/ml yielded no mitotic cells (see Appendix I, Table 1, attached). In the second range-finding study, dose-dependent decreases in mitotic index and increases in average cell generation times were observed. Similar cytotoxicity was seen at the 20 ug/ml dose level as was observed in the first experiment. The ACGT at 15 ug/ml with and without activation was lengthened to 16.2 and 17.6 hours, respectively. Based on this, the highest concentration of Propachlor tested was 15 ug/ml, both with and without metabolic activation. The Propachlor levels of 5, 10, and 15 ug/ml were chosen as the highest three scorable doses.

2. Chromosomal Aberration Assay Without Metabolic Activation - There was a decrease in mitotic index for 15 ug/ml doses at the 12- and 36-hour times, indicating that cytotoxic levels were tested. No statistically significant increases in the percentage of cells with structural aberrations per cell were reported at any treatment level of Propachlor, regardless of harvest times. The positive control, MMS at 50 ug/ml, demonstrated the adequacy of the assay to detect clastogens (see Appendix I, Table 2, attached).

3. Chromosomal Aberration Assay With Metabolic Activation - There was a treatment-related decrease in mitotic index reported at 15 ug/ml, indicating testing was at cytotoxic levels of test material. A statistically significant increase in the percent of cells with structural aberrations was observed for the 15 ug/ml dose group at the 12-hour harvest time. Additionally, a statistically significant increase was reported in structural aberrations per cell for this group at 12- and 24-hour harvest times; however, the increase was not dose-related (see Appendix I, Table 4). The positive control,

CP at 20 and 50 ug/ml, yielded the expected positive response, thus demonstrating the adequacy of the assay to detect clastogens (see Appendix I, Table 3).

E. CONCLUSION

There was a weak but statistically significant response at the 15 ug/ml dose level of Propachlor with metabolic activation at the 12- and 24-hour harvest times. There was no apparent dose-response relationship. According to the authors, Propachlor is negative in the assay without metabolic activation and a possible weak clastogen in the assay with metabolic activation. TB agrees with this conclusion. In light of the positive activity, it will assumed that Propachlor is positive in this assay despite the lack of documentation detailing the nature and grade of Propachlor used.

REFERENCES

Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P. , and Zeiger, E. Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutag. 7, 1-51 (1985).

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DATA EVALUATION REPORT

CHEMICAL: Propachlor Tox. Chem. No.: 194

STUDY TYPE: Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures

ACCESSION NUMBER: 257647

SYNONYMS/CAS No.: Ramrod®/1918-16-7

SPONSOR: Monsanto Company

TESTING FACILITY: SRI International

TITLE OF REPORT: Evaluation of the Potential of Propachlor to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures

AUTHOR(S): Karen L. Steinmetz and Jon C. Mirsalis, Ph.D.

STUDY NUMBER: SR-84-239; SRI Project No. LSC-7538

REPORT ISSUED: December 10, 1984

QUALITY ASSURANCE: A quality assurance statement was provided.

Classification: Unacceptable, pending clarification of whether the test material used is the technical grade of Propachlor.

CONCLUSION(S) - Executive Summary: Under the conditions of this assay, Propachlor was not shown to be genotoxic in the *in vitro* rat hepatocyte DNA repair assay at concentrations up to 25 ug/ml (higher concentrations were cytotoxic).

A. MATERIALS

1. Test Material: Name: 2-chloro-N-(1-methylethyl)-N-phenylacetamide; (Propachlor); Description: Dark-tan solid; Batch #: not provided; Purity: 95.8%; Solvent: acetone; Solubility: limit - 5000 ug/ml acetone.

2. Control Materials: Negative: an untreated medium control and a solvent control were used. The final concentration of acetone was maintained at 1% to preclude the possibility of a cytotoxic effect due to the solvent. Positive: 2-acetylaminofluorene (2-AAF; 0.5 ng/ml).

3. Test Organism: Primary hepatocytes were isolated from the livers of 2 adult male Fischer-344 rats (Simonsen Laboratories, Gilroy, CA), which were allowed to attach to glass cover slips. Livers were perfused with a collagenase solution (procedure of Williams, 1977) and inoculated into culture dishes containing coverslips in Williams' Medium E supplemented with 2 mM glutamine, 50 ug/ml gentamicin, and 10% fetal bovine serum. Viable cells were used immediately and all subsequent steps were performed in serum-free medium.

4. Test Material Concentrations: The test material (and positive control) was diluted in acetone immediately prior to assay, which when diluted in culture medium, yielded the following concentrations: 0.1, 0.5, 1.0, 5.0, 10, 25, 50, 100, 500, 1000, and 5000 ug/ml.

B. TEST PERFORMANCE

In the preliminary assay, three cultures were used for each of the ten dilutions of Propachlor, and for each of the controls. Approximately 2 hours after seeding, the cultures were exposed simultaneously to the test (or control) material and to 10 uCi/ml ^{3}H-thymidine (specific activity - 80 Ci/mmole) for 18-19 hours. Following exposure, all cultures were washed with medium, swelled in hypotonic solution, fixed, and washed with water. The coverslips were mounted on slides, dipped in Kodak NTB-2 emulsion, and exposed at -20°C for 7 days prior to development. Cells were stained in methyl-green Pyronin Y. The UDS assay was repeated at five non-cytotoxic concentrations of Propachlor, the highest concentration, chosen by the Sponsor, was 50 ug/ml.

Quantitative autoradiographic grain counting was performed. Fifty morphologically unaltered cells on a randomly selected area of the slide were counted. The highest count from two nuclear-size areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains/nucleus (NG). The percentage of cells in repair was calculated as the percentage of cells with at least 5 NG. One hundred and fifty cells were scored for each concentration reported for each experiment.

C. ANALYSIS OF DATA

1. Statistical Methods: VAX 11/782 computer programs were used. Frequency distributions of grain counts for each test concentration as well as average and median grain counts were calculated and compared with control values.

2. Criteria for Positive and Negative Results: If UDS (amount of ^{3}H-thymidine incorporated) is markedly (not further defined) elevated above the solvent control value, the compound is considered positive. If testing has been to the limits of solubility or cytotoxicity of the test material, or 5000 ug/ml, and UDS is not significantly elevated above the solvent control value, the compound is considered negative. The presence of a dose response, a change in the frequency distribution of cellular responses, an increase in the percentage of cells in repair, and reproducibility of the data were all said to be taken into consideration in classifying the test material.

D. RESULTS

The results were presented in Table 1, attached. Cytotoxicity was observed at 50 ug/ml and above in the preliminary experiment and also at 50 ug/ml (HDT) in the replicate experiment. A precipitate was reported at 5000 ug/ml. In the preliminary assay, concentrations utilized ranged from 0.1 to 10 ug/ml, and in the replicate assay, from 0.5 to 25 ug/ml. The net grain counts were negative at each of the test material concentrations and in the solvent control. A strong positive response was produced by 2-AAF in both experiments; i.e., 36.7 and 24.4 NG.

E. CONCLUSION

TS agrees with the authors that Propachlor was not shown to be genotoxic, under the conditions of this assay, in the in vitro rat hepatocyte DNA repair assay. Note: Acetochlor (an analogue) was reported in Peer Review document as negative in this test; however, alachlor (another analogue) is reported in a Peer Review document to be weakly genotoxic in the in vivo-in vitro modification of this test.

Before a final assessment can be made on this study, clarification of whether the test material used is the technical grade of Propachlor is required.

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