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

OCT 16 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Propachlor - Report of the Cancer Assessment Review Committee

FROM: Jess Rowland 10/9/97.
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

Through: William Burnam
Chairman
Cancer Assessment Review Committee
Health Effects Division (7509C)

To: Mike Metzger
Chief, Reregistration Action Branch 1
Health Effects Division (7509C)

And

Jim Tompkins, PM 25
Fungicide-Herbicide Branch
Registration Division (7505C)

The Cancer Assessment Review Committee met on July 30, 1997 to evaluate the carcinogenic potential of Propachlor. Attached please find the Final Cancer Assessment Document.

cc: Margaret Stasikowski
Stephanie Irene
Randy Perfetti
Kathryn Boyle
Whang Phang
File

FINAL

FINAL

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF ***PROPACHLOR***

FINAL REPORT

September 30, 1997

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Linda Taylor, Ph.D., Toxicologist

DOCUMENT PREPARATION: Jess Rowland,M.S., Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam, Chairman

Karl Baetcke

Marion Copley

Kerry Dearfield

Virginia Dobozy

Mike Ioannou

Nancy McCarroll

Hugh Pettigrew

Esther Rinde

Joycelyn Stewart

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Luke Brennecke, Pathology Consultant

Lori Brunsman, Statistical Analysis — H. Pettigrew for Lori Brunsman

OTHER ATTENDEES: Kathryn Boyle, Alberto Protzel, Whang Phang

CONTENTS

EXECUTIVE SUMMARY

On July 30, 1997, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Propachlor.

Dr. Linda Taylor introduced the chronic toxicity/carcinogenicity studies in F344 and Sprague Dawley rats and CD-1 mice by: describing the experimental design; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data , the adequacy of the dose levels tested; and presenting the weight of the evidence for the carcinogenicity of Propachlor. Dr. Taylor also discussed the toxicology, metabolism and mutagenicity studies as well as structure-activity relationships.

Propachlor was administered in the diet: to male and female Fischer-344 rats at 0, 100, 300, 1000 or 2500 (male) and 5000 (female) ppm for 24 months; to male and female Sprague-Dawley rats at 0, 10, 50 or 500 ppm for 24 months; and to male and female CD-1 mice at 0, 100, 500, 1500 or 6000 ppm for 18 months. The Committee concluded that the dose levels tested in the Fischer 344 rat and the CD-1 mouse studies were adequate to assess the carcinogenic potential of Propachlor, but the dose levels tested in the Sprague-Dawley rats were less than adequate to assess carcinogenicity. Tumors were seen in the stomach of male Fischer 344 rats, the thyroid glands of male and the ovaries of female Sprague-Dawley rats, as well as the liver of male CD-1 mice.

In Fischer 344 rats, a single carcinoma of the glandular stomach was seen in one male at the high dose (2500 ppm or 125.3 mg/kg/day). This tumor was attributed to treatment since:

(i) stomach tumors are rare in rats;

(ii) the incidences and severity of the non-neoplastic lesions (herniated mucosal glands, mucosal hyperplasia of the pylorus and pyloric cysts) observed only in treated rats of both sexes increased with dose in males;

(iii) no stomach tumors were observed in historical control rats (250 males and 250 females) at the testing laboratory; and

(iv) stomach tumors were also seen in rats treated with structural analogs (Alachlor and Butachlor).

The Committee noted that the stomach tumor seen in this study was of a different cell type from the stomach tumors induced by the analogs. Alachlor induced osteosarcomas, malignant mixed gastric tumors and combined gastric adenocarcinomas and/or malignant mixed gastric tumors in both sexes of Long-Evans rats. Butachlor induced poorly differenciated carcinoids of the fundus in female Sprague-Dawley rats.

In the Sprague-Dawley rats, C-cell adenomas, carcinomas and combined adenomas/carcinomas of the thyroid glands were observed in both sexes of rats including the controls. The thyroid tumors were attributed to treatment for the following reasons:

(i) there were significant positive trends for adenomas (males, p=0.039; females, p= 0.048) and combined adenomas/carcinomas (males, p=0.012; females, p=0.037);

(ii) the incidence of combined adenomas/carcinomas in males (6/47, 13%) at the high dose (23.88 mg/kg/day) was significantly (p= 0.047) increased when compared to controls (1/50, 2%);

(iii) the incidence in females at the high dose (5/47, 11%), although not statistically significant, was higher than the controls (3/45, 7%); and

(iv) thyroid tumors were also seen with structural analogs (Acetochlor, Alachlor and Butachlor).

Also in the Sprague-Dawley rats, ovarian granulosa/theca cell (benign and combined benign/ malignant) tumors were seen and were attributed to treatment for reasons stated below:

(i) there were significant positive trends for both the benign (p=0.005) and combined benign/malignant (p=0.002) tumors;

(ii) the incidence of combined benign/malignant tumors at the high dose was significantly (p=0.033) increased (pair-wise) when compared to controls;

(iii) the incidences at the high dose (23.88 mg/kg/day) were higher for all three types [benign, 4/47 (9%); malignant, 1/47 (2%); and combined 5/47 (11%] when compared to control (0/44, 0%); and

(iv) the incidences exceed the historical control range of 0 - 5%.

In CD-1 mice, the hepatocellular tumors seen in males at the high dose (6000 ppm or 847.3 mg/kg/day) were attributed to treatment because:

(i) there were significant positive trends for all three types (adenomas, p=0.000, carcinomas, p=0.002 and combined adenomas/carcinomas, p=0.000);

(ii) adenomas (29/40, 72.5%) and combined adenomas/carcinomas (31/49, 63%) were significantly (p=0.000) increased compared to controls (0/49, 0%); and

(iii) the incidences exceeded the historical control range for adenomas (0-21.7%) as well as combined adenomas/carcinomas (5.2 - 25%).

In mutagenicity testing, the *in vitro* clastogenic activity of Propachlor was consistent with the results obtained in similar testing with structural analogs (Acetochlor, Alachlor, and Diemethenamid (SAN 582H).

Propachlor is structurally related to Acetochlor, Alachlor, Butachlor, Metolachlor and Dimethenamid. In rats, Propachlor induced tumors at the same sites (stomach and thyroids) as Acetochlor (thyroids), Alachlor (stomach/thyroids) and Butachlor (stomach/thyroids), but it did not induce either liver tumors seen with Metolachlor and Dimethenamid or nasal tumors seen with Acetochlor, Alachlor and Butachlor. In mice, the hepatocellular tumors seen with Propachlor were seen with Acetochlor.

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified Propachlor as a **"likely"** human carcinogen by all routes of exposure. The weight-of-the-evidence for this classification are as follows:

(i) the observance of multiple tumors at multiple sites including the rare stomach tumor in male Fischer 344 rats, thyroid tumors and ovarian granulosa/theca cell tumors in male and female Sprague-Dawley rats, respectively and hepatocellular tumors in male CD-1 mice;

(ii) *in vitro* clastogenic activity;

(iii) tumors seen at one or more of the same sites with three structurally related chloracetanilide compounds;

(iv) lack of data on mode of actions; and

(v) the relevance of the observed tumors to human exposure.

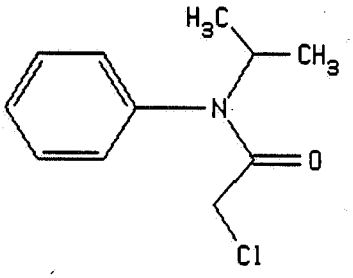
The Committee recommended a linear low-dose approach for human risk characterization and extrapolation of risk should be based on both the neoplastic (ovarian tumors in rats and liver tumors in male mice) and non-neoplastic (liver hypertrophy in mice) lesions. The "points of departure" for extrapolations are 50 ppm (2.4 mg/kg/day) for neoplastic lesions (ovarian tumors) and 500 ppm (75 mg/kg/day) for non-neoplastic lesions (liver hypertrophy).

I. INTRODUCTION

On July 30, 1997, the Health Effects Division's Cancer Assessment Review Committee evaluated the carcinogenic potential of Propachlor. The Committee evaluated a combined chronic toxicity/ carcinogenicity study in Fischer 344 rats, a combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats and a carcinogenicity study in CD-1 mice. Dr. Linda Taylor of the Reregistration Branch-1 presented the experimental design and results of these studies, statistical analysis of the tumor data, weight of the evidence considerations, as well as the toxicology, metabolism, mutagenicity and structure-activity data of Propachlor.

II. BACKGROUND INFORMATION

Propachlor (2-chloro-N-isopropylacetanilide) is an alpha-chloroacetamide registered for use as an herbicide on corn and sorghum, and onion seed in Oregon and Washington. The CAS Registry Number is 1918-16-7. The PC Code is 019101. The Tox. Chemical No. is 194.



Propachlor

III. EVALUATION OF CARCINOGENICITY STUDIES

1. *Combined Chronic Toxicity/Carcinogenicity Study with Propachlor in F-344 Rats*

Reference: Combined Chronic/Oncogenicity Study of Propachlor Administered in Feed to F-344 Rats for 24 Months (1996). Monsanto Study No. ML-93-190, Laboratory Project No. EHL 93075, MSL No. 14861, RD 1363. dated 11/26/96. **MRID 44168301.**

A. Experimental Design

Propachlor (97.83% a.i.) was administered in the diet to Fischer 344 rats (60/sex/dose) at dose levels of 0, 100, 300, 1000, or 2500 (males)/ 5000 (females) ppm (0, 5.4, 16.1, 53.6 and 125.3 mg/kg/day in males and 0, 6.4, 19.3, 65.5, and 292.1 mg/kg/day in females, respectively) for 24 months.

B. Discussion of Tumor Data

Except for a glandular carcinoma of the stomach in one male rat at the high dose (2500 ppm or 125.3 mg/kg/day), no treatment-related tumors were seen in males. There was no apparent treatment-related increase in tumors in the treated female rats when compared to the control rats.

When compared to historical control data, no stomach tumors were found in 5 two-year studies consisting of a total of 250 males and 250 female rats that have been performed at the testing facility. The gross pathology data from each of these studies was examined and every lesion described as a nodule or mass was sectioned, processed, and examined microscopically. The Registrant stated that the stomach tumor found in the present study was unlike the gastric tumor type produced by other chloroacetanilides, which arise as a consequence of mucosal atrophy, including atrophy of the parietal or acid-producing cells of the stomach. The stomach tumors observed following exposure to the other chloroacetanilides were said to consist of neuroendocrine and glandular components, and they only occurred in the fundic region of the stomach [the references on these tumors are for Sprague-Dawley rats]. The Registrant pointed out that the Propachlor stomach tumor arose in the pyloric region of the stomach and had no "neuroendocrine component, and the phenotype was distinctly different from that which is characteristic of tumors produced by other chloroacetanilides." It was also stated that "there was no mucosal/parietal cell atrophy, which is requisite for the induction of gastric neoplasia by the chloroacetanilides."

C. Non-Neoplastic Lesions in the Stomach

(i) STOMACH - Herniated mucosal glands (submucosa/tunica muscularis), mucosal hyperplasia of the pylorus, and pyloric cyst(s) were observed only in treated rats of both sexes, and the incidence and severity increased with dose in males (Table 1). Females at the 1000 ppm dose level and both sexes at the highest dose level also displayed erosion/ulceration of the glandular mucosa of the stomach (Table 2).

Table 1. Mean Severity of Stomach Lesions in Male Rats Fed Propachlor.

Lesion / Dose (ppm)	0	100	300	1000	2500
herniated mucosal glands	0.0	0.0	1.0	2.0	2.7
pyloric hyperplasia	0.0	0.0	2.0	1.5	2.5

Table 2. Non-Neoplastic Lesions of the Stomach in F344 Rats Fed Propachlor

Lesions	0	100	300	1000	2500/50
MALES No. Examined =	**59**	**60**	**60**	**60**	**60**
cyst(s), pylorus	0	0	0	3	34**
erosion, fundus	2	14**	6	9	4
erosion/ulceration, pylorus	8	14	13	13	32**
herniated mucosal glands, submucosa/tunica muscularis	0	0	1	6	39**
infiltrate, mononuclear cell	22	19	18	9	27
mineralization	2	18**	1	3	2
mucosal hyperplasia, pylorus	0	0	1	4	29**
necrosis, squamous mucosa	0	0	0	0	2
osseous/cartilaginous metaplasia	0	0	0	0	3
FEMALES No. Examined =	**60**	**"-"**	**60**	**59**	**60**
cyst(s), pylorus	0	-	0	0	27**
erosion, fundus	6	-	5	6	4
erosion/ulceration, pylorus	5	-	0	11	35**
herniated mucosal glands, submucosa/tunica muscularis	0	-	0	0	41**
infiltrate, mononuclear cell	5	-	14	5	0
mineralization	1	-	5	0	0
mucosal hyperplasia, pylorus	0	-	0	0	34**

"-" = Not examined
** = $p < 0.01$

D. Non-neoplastic and Neoplastic Lesions in the Liver, Thyroid Glands and Nose/Turbinates

(i) Liver - The incidences of non-neoplastic and neoplastic lesions are presented in Table 3. The incidence and severity of hepatocellular hypertrophy (centrilobular/ midzonal) were increased in a dose-related manner in both sexes. There was no increase in the incidence of hepatocellular tumors in either sex.

Table 3. Lesions of the Liver in F344 Rats Fed Propachlor

Lesion/Sex/Dose	0 ppm	100 ppm	300 ppm	1000 ppm	2500/5000 ppm
MALES No. Examined =	60	60	60	60	60
hepatocellular hypertrophy centrilobular/midzonal	1	0	6	31**	49**
eosinophilic focus	8	7	13	23**	21*
lipidosis	1	3	2	5	8
hepatocellular atrophy, random	2	0	2	3	2
hepatocellular hypertrophy, panlobular	1	0	0	0	0
hepatocellular vacuolation	10	9	10	11	11
hepatocellular adenomas	1	1	1	3	2
hepatocellular carcinomas	0	0	0	0	1
FEMALES No. Examined =	60	60	60	59	60
hepatocellular hypertrophy centrilobular/midzonal	0	0	11**	20**	55**
eosinophilic focus	2	6	16**	15**	10
lipidosis	0	8*	5	10**	8*
hepatocellular atrophy, random	0	1	1	1	1
hepatocellular hypertrophy, panlobular	1	2	0	0	0
hepatocellular vacuolation	19	18	19	29	2
hepatocellular adenomas	0	0	0	2	0

* $p<0.05$; ** $p<0.01$

(ii) Thyroid Glands - The incidence of several non-neoplastic and neoplastic lesions of the thyroid glands is listed in Table 4. Follicular cell adenoma/cystadenoma was observed only in the treated rats, with the highest incidence occurring at the highest dose level in both sexes.

Table 4. Lesions of the Thyroid Glands in F-344 Rats Fed Propachlor

Lesions/Sex/Dose	0 ppm	100 ppm	300 ppm	1000 ppm	2500/500 ppm
MALES No. Examined =	59	0	60	60	60
hyperplasia, follicular epithelium	3	-	0	1	6
hyperplasia, C cell	9	-	17	20	8
cyst(s), follicular	0	-	4	2	1
cyst(s), ultimobranchial	0	-	1	0	4
C-cell adenoma	2	-	7	9	4
follicular cell adenoma/cystadenoma	0	-	1	1	2
C-cell carcinoma	8	-	5	2	3
follicular cell carcinoma	1	-	0	3	2
FEMALES No. Examined =	60	0	60	59	60
hyperplasia, follicular epithelium	0	-	0	1	1
hyperplasia, C-cell	6	-	13	11	6
cyst(s), follicular	1	-	0	0	2
cyst(s), ultimobranchial	1	-	1	1	3
C-cell adenoma	2	-	4	7	5
follicular cell adenoma/cystadenoma	0	-	0	0	1
C-cell carcinoma	2	-	4	3	2

(iii) NOSE/TURBINATES - A single chondroma was observed in one male at the 300 ppm dose level and one male at 2500 ppm .

E. Adequacy of the Dosing for Assessment of Carcinogenicity

There was no adverse effect on survival observed for either sex. All dose groups and controls had ≥66% survival at study termination. Decreased body weight was observed at the highest dose level in both sexes throughout the study (males, 93% /females, 72% of control at study termination), and this was accompanied by decreased food intake. Because of the poor palatability of Propachlor, in order to attain a sufficiently high dose level of Propachlor, the high dose for each sex had to be increased from 1000 ppm over several weeks (high-dose groups started at 1000 ppm and were increased weekly by 500 ppm until targeted dose was attained; 2500 ppm in males and 5000 ppm in females). Body-weight gains over the first 90-day period for the highest dose groups were 82% and 79% of control for the males and females, respectively. In addition to the decreased body-weight gain for both sexes (males, 91%/females, 58% of control overall) and the decreased body weight throughout study (males, 94%/ females, 71% of control at termination). Histopathology revealed increased incidence of stomach and liver lesions. The NOEL was 100 ppm (5.4 and 6.4 mg/kg/day, in males and females, respectively), and the LOEL was 300 ppm (16.1 and 19.3 mg/kg/day, in males and females, respectively), based on stomach lesions in males and liver lesions in both sexes. The highest dose tested for each sex (2500 and 5000 ppm, for males and females, respectively) is considered to be adequate to assess the carcinogenicityl of Propachlor.

2. Combined Chronic Toxicity/Carcinogenicity Study in Sprague-Dawley Rats

Reference: Combined Chronic Toxicity and Oncogenicity Study of Propachlor in Rats (Propachlor). (1987). Study No. HL-83-350/241-160, dated 8/14/87. **MRID 40473101.**

A. Experimental Design

Propachlor (96.1% a.i.) was administered in the diet to Sprague-Dawley CD®-Crl:CD(SD)BR rats (60/sex/dose) at dose levels of 0, 10, 50, or 500 ppm (0, 0.48, 2.39, and 23.88 mg/kg/day in males and 0, 0.60, 3.04, or 30.05 mg/kg/day, in females, respectively) for 104 weeks. There was an interim sacrifice at 53 weeks of 10 of these rats/sex/group.

B. Discussion of Tumor Data

As shown in Table 5, male rats had significant increasing trends in thyroid C-cell adenomas and adenomas and/or carcinomas combined. When compared to controls, there was a significant (pair-wise test) increase in combined C-cell adenomas and/ or carcinomas at the high dose (500 ppm). Historical control data from the testing laboratory were not available.

Among female rats, there were significant increasing trends in thyroid C-cell adenomas and combined adenomas and/or carcinomas (Table 6) as well as benign ovarian granulosa/theca cell tumors and combined benign and/or malignant tumors (Table 7). There was a significant difference in the pair-wise comparison of the 500 ppm dose group with the controls for the combined ovarian granulosa/theca cell benign and/or malignant tumors (Table 7).

Table 5. Male Rats: Thyroid C-Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

Tumor Type	0 ppm	10 ppm	50 ppm	500 ppm
Adenoma	1[a]/50	1/49	1/49	4/47
%	2	2	2	9
p =	0.039*	0.748	0.748	0.162
Carcinoma	0/50	1[b]/49	1/49	2/47
%	0	2	2	4
p =	0.110	0.495	0.495	0.232
Combined	1/50	2/49	2/49	6/47
%	2	4	4	13
p =	0.012*	0.492	0.492	**0.047***

[+] Number of tumor bearing rats/Number of rats examined, excluding those that died or were sacrificed before Week 54.

[a] First adenoma observed at Week 105, dose 0 ppm.

[b] First carcinoma observed at Week 98, dose 10 ppm.

Note: Interim sacrifice rats are not included in this analysis. There were no thyroid C-cell tumors in any interim sacrifice rats.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Female Rats: Thyroid C-Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

Tumor Type	0 ppm	10 ppm	50 ppm	500 ppm
Adenoma	1[a]/45	0/49	0/48	3/47
%	2	0	0	6
p =	0.048*	0.479[n]	0.484[n]	0.325
Carcinoma	2[b]/45	0/49	1/48	2/47
%	4	0	2	4
p =	0.288	0.227[n]	0.476[n]	0.675
Combined	3/45	0/49	1/48	5/47
%	7	0	2	11
p =	0.037*	0.106[n]	0.284[n]	0.382

[+] Number of tumor bearing rats/Number of rats examined, excluding those that died or were sacrificed before Week 54.

[a] First adenoma observed at Week 98, dose 0 ppm.

[b] First carcinoma observed at Week 105, dose 0 ppm.

[n] Negative change from control.

Note: Interim sacrifice rats are not included in this analysis. There were no thyroid C-cell tumors in any interim sacrifice rats.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$

Table 7. Female Rats: Ovarian Granulosa/Theca Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

Tumor Type	0 ppm	10 ppm	50 ppm	500 ppm
Benign	0/44	0/49	1/48	4[a]/47
%	0	0	2	9
p =	0.005**	1.000	0.522	0.067
Malignant	0/44	0/49	0/48	1[b]/47
%	0	0	0	2
p =	0.250	1.000	1.000	0.517
Combined	0/44	0/49	1/48	5/47
%	0	0	2	11
p =	0.002**	1.000	0.522	**0.033***

[+] Number of tumor bearing rats/Number of rats examined, excluding those that died or were sacrificed before Week 54.

[a] First benign tumor observed at Week 89, dose 500 ppm.

[b] First malignant tumor observed at Week 106, dose 500 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no ovarian granulosa/theca cell tumors in any interim sacrifice rats.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data from the testing laboratory were not available. From published data on the incidence of granulosa cell and theca cell tumors in Sprague-Dawley rats [Charles River], the incidence ranged from zero out of 70 to 3 out of 59. The majority of the studies showed one or zero. For the thyroid, the author stated that the incidence was within the historical control, although no data were provided.

C. Non-neoplastic findings: Follicular cell hyperplasia was seen only in females at 10 ppm (1/60) and 50 ppm (1/59). The incidence of C-cell hyperplasia is presented in Table 8.

Table 8. C-Cell Hyperplasia of the Thyroid Glands in Sprague-Dawley Rats Fed Propachlor.

Lesions	0 ppm	10 ppm	50 ppm	500 ppm
Males				
No. Examined	60	60	60	58
No. Observed	9	2	1	6
Females				
No. Examined	60	60	59	60
No. Observed	4	2	4	6

D. Adequacy of Dosing for Assessment of Carcinogenicity

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Propachlor in either male or female rats. Comparable body weights were observed in the male groups and only slightly lower body weights were observed in the high-dose females compared to the control. Body-weight gains were ≈92% of control for the high-dose females during the first 90-day interval. Food consumption was comparable among the groups for both sexes. In the 90-day rat study at palatable dose levels of 300 ppm and 1500 ppm, decreased body weights were observed in the males (90% of control) and females [92% of control] at 1500 ppm, but no other effects were observed in either sex. At the high-dose level of 7500 ppm in that study, the effects observed were attributed to the poor palatability of Propachlor and the resulting decrease in food consumption and lack of adequate nutrition. The dose levels tested in this study were not considered high enough to assess the carcinogenic potential of Propachlor.

3. Carcinogenicity Study in Mice

Reference: Oncogenicity Study of Propachlor Administered in Feed to CD-1® Mice for 18 Months. Monsanto Study No. ML-93-191; Project No. EHL 93076, dated 7/19/96; **MRID 44069801**; Document No. 012097.

A. Experimental Design

CD-1 mice (60/sex/dose) were fed diets containing Propachlor at dose levels of 0, 100 ppm (♂ 14.6/♀ 19.3 mg/kg/day), 500 ppm (♂ 75.0/♀ 100 mg/kg/day), 1500 ppm (♂ 222.9/♀ 276.7 mg/kg/day), or 6000 ppm (♂ 847.3/♀ 1006.9 mg/kg/day) for 18 months.

B. Discussion of Tumor Data

As shown in Table 9, treatment-related increases in liver tumors were observed only in male mice. Males exhibited significant increasing trends as well as significant differences in the pair-wise comparisons at the 6000 ppm dose group when compared to controls for hepatocellular adenomas and adenomas and/or carcinomas combined. There was also a significant trend in hepatocellular carcinomas. Two of the males with hepatocellular carcinoma at the highest dose level also displayed hepatocellular adenomas, and the one high-dose male with hepatoblastoma also displayed an adenoma. Twenty-four of the 30 male mice with hepatocellular adenomas also displayed hypertrophy. Of the 4 males at the 1500 ppm dose levels with adenomas, 3 also displayed hepatocellular hypertrophy. There were no treatment-related tumors observed in female mice.

When compared to historical controls of the testing facility, the incidence of hepatocellular adenoma in the current study exceeded that of the historical control. The incidence of carcinomas was within that observed in the historical control, but when combined with the adenomas, the incidence exceeded the historical controls. The highest incidence of adenoma observed in the historical control data was 13/60 (21.7%) for male mice examined in one study conducted in 1993. Of the 15 studies in the historical control data, 7 were performed between 1990 and 1994; the other 8 were performed between 1981 and 1988. The incidence of liver tumors in the females was within that of the historical control, although no carcinomas and only 1 adenoma/study were observed in the 7 studies in the historical control performed during the 1990-94 interval.

C. Non-Neoplastic Lesions

The non-neoplastic lesions observed in the liver and stomachs of both sexes of mice are presented in Table 10.

Table 9. Male Mice: Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results

Tumor Type	0 ppm	100 ppm	500 ppm	1500 ppm	6000 ppm
Adenomas	2/49	3/50	2/50	4/48	**29[a]/40**
%	4	6	4	8	72.5
p =	0.000**	0.510	0.684	0.329	**0.000****
Carcinomas	0/49	0/50	0/50	1/48	4[b]/49
%	0	0	0	2	8
p =	0.002**	1.000	1.000	0.495	0.059
Combined	2/49	3/50	2/50	5/48	**31[c]/49**
%	0	6	4	10	63
p =	0.000*	0.510	0.684	0.209	**0.000****

[+] Number of tumor bearing mice/Number of mice examined, excluding those that died or were sacrificed before Week 55.

[a] First adenoma observed at Week 54, dose 6000 ppm, in an interim sacrifice mouse. Second adenoma observed at Week 67, dose 6000 ppm, in a mouse that died on study.

[b] First carcinoma observed at week 68, dose 6000 ppm.

[c] Two mice in the 6000 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice mice are not included in this analysis. One mouse in the 6000 ppm dose group of the interim sacrifice group had a hepatocellular adenoma.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$

Table. 10. Non-Neoplastic Lesions of the Liver and Stomach in CD-1 Mice Fed Propachlor.

Lesion/Sex/Dose	0 ppm	100 ppm	500 ppm	1500 ppm	6000 ppm
Males - Liver No.Examined =	60	60	60	60	60
hepatocellular hypertrophy, centrilobular/midzonal	2	1	4	30**	47**
individual hepatocytes, necrosis	0	1	3	2	15**
eosinophilic focus	0	1	0	9**	26**
mononuclear cell infiltrate, random	20	15	12	14	12
telangiectasis	0	0	1	0	13**
pigment deposition, Kupffer cells	0	0	0	2	22**
hepatocellular degeneration/necrosis, centrilobular	1	0	0	2	4
hepatocellular degeneration/necrosis, random	0	0	2	4	3
Females - Liver No.Examined =	60	**10**	60	60	60
hepatocellular hypertrophy, periportal	0	0	0	3	51**
mononuclear cell infiltrate, random	22	2	15	12	34
hepatocellular degeneration/necrosis, random	2	0	7	3	1
hepatocellular hypertrophy, centrilobular/midzonal	0	1	0	0	2
pigment deposition, Kupffer cells	1	0	1	2	12**
individual hepatocytes, necrosis	0	0	1	0	1
Males - Stomach No. Examined =	60	0	58	60	59
inflammation, glandular mucosa	4	-	1	2	6
erosion/ulceration, glandular mucosa	0	-	0	0	8**
hyperplasia, glandular, mucosa	6	-	7	9	9
herniated mucosal glands, submucosa/tunica muscularis	1	-	0	7	13**
Females- Stomach No. Examined =	9	0	59	60	60
inflammation, glandular mucosa	4	-	1	1	2
erosion/ulceration, glandular mucosa	1	-	6	2	2
hyperplasia, glandular, mucosa	8	-	7	2	3
herniated mucosal glands, submucosa/tunica muscularis	0	-	0	0	6*

* = $p < 0.01$;

D. Adequacy of Dosing for Assessment of Carcinogenicity

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Propachlor in either male or female mice (Tables 11 and 12, respectively, for males and females). This study had a ramping phase of the dosing procedure in which the high-dose mice were fed initially a concentration of 1500 ppm, which was increased weekly by 500 ppm until the diets contained 6000 ppm. Dosing was considered to be adequate for assessing the carcinogenic potential of Propachlor based on the decreases in food consumption and a concomitant decrease in body-weight gain.

Table 11 Male Mice: Mortality Rates[+] and Cox or Generalized K/W Test Results.

Dose (ppm)	Study Week				
	1-26	27-53	54[i]	54-79[f]	TOTAL
0	0/60	1/60	10/59	4/49	5/50 (10%)
100	0/60	0/60	10/60	3/50	3/50 (6%)
500	0/60	0/60	10/60	12/50	12/50 (24%)
1500	1/60	1/59	10/58	5/48	7/50 (14%)
6000	0/60	1/60	10/59	9/49	10/50 (20%)

[+] Number of mice that died during interval/Number of mice alive at the beginning of the interval.

[i] Interim sacrifice at Week 54.

[f] Final sacrifice at Week 78.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 12 Female Mice: Mortality Rates[+] and Cox or Generalized K/W Test Results.

Dose (ppm)	Study Week				
	1-26	27-53	54[i]	54-79[f]	TOTAL
0	0/59[a]	1/58[b]	10/57	7/47	8/48 (17%)
100	0/60	0/60	10/60	10/50	10/50 (20%)
500	1/60	0/59	10/59	7/49	8/50 (16%)
1500	1/59[c]	1/59	10/58	8/48	9/49 (18%)
6000	0/60	2/60	10/58	9/48	11/50 (22%)

[+] Number of mice that died during interval/Number of mice alive at the beginning of the interval.

[i] Interim sacrifice at Week 54.

[f] Final sacrifice at Week 78.

[a] One accidental death at week 13, dose 0 ppm.

[b] One accidental death at week 28, dose 0 ppm.

[c] One accidental death at week 3, dose 1500 ppm

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

4. Carcinogenicity Study in Mice

Reference: Oncogenicity Study in Mice. HL-83-349; Project No. 241-159, dated January 19, 1987; **MRID 40162501**; Document No. 006677.

A. Experimental Design

CD-1 mice (60/sex/group) were fed Propachlor at dose levels of 0, 10 (♂ 1.62/ ♀2.01), 50 (♂ 8.12/♀ 10.03), or 500 ppm (♂ 81.25/♀ 104.89) for 78 weeks.

B. Discussion of Tumor Data

There were no treatment-related effects observed on survival, body weight/gain, food consumption, hematology, gross pathology, or histopathology in either sex, and there were no apparent treatment-related increases in tumors in either sex compared to the controls. The dose levels utilized in this study were determined to be inadequate (not high enough) for assessing the carcinogenic potential of Propachlor.

IV. TOXICOLOGY

1. Metabolism

In a metabolism study in rats in which single doses of 25 mg Propachlor/kg of body weight were administered orally, 91% of the dose was recovered in 56 hours, with 68% of the dose being excreted in urine, 10% in the feces, and 4% found in the carcass. Eleven metabolites were identified. The metabolic fate of Propachlor depends to a large extent on the presence of the intestinal microflora. Propachlor metabolites can make 3 or more cycles in the enterohepatic circulation. In the first cycle, Propachlor is metabolized via the mercapturic acid pathway and the conjugates are excreted in the bile. The second cycle is initiated when the biliary mercapturic acid pathway metabolites are metabolized by a microflora C-S lyase to reabsorbable metabolites, which are then metabolized to glucuronides that are secreted with the bile. Subsequent cycles result from microfloral β-glucuronidase activity. Propachlor appears to undergo rapid absorption, distribution, metabolism, and excretion with little, if any, tissue retention in rats. From the studies available, it can be stated that, following initial glutathione conjugation, metabolism proceeds primarily via the mercapturic acid pathway with concurrent oxidative reactions and glucuronic acid conjugation. Initially-formed metabolites undergo extensive excretion and enterohepatic circulation. (**MRID Nos: 00157496 through 00157500 and 00157502 through 00157507).**

2. Mutagenicity:

a) In a Chinese hamster ovary (CHO) cell forward mutation (HGPRT) assay, Propachlor was mutagenic based on a concentration-dependent increase in mutant frequency to over a doubling of ethanol control frequency at 50 µg/mL with 5% S9. This was supported by appropriate toxicity at this concentration, an increase in absolute colony numbers, and the relatively tight spontaneous background reported in the testing lab **(MRID No. 00153939)**.

b) In a CHO/chromosomal aberration assay, Propachlor was found to induce a clastogenic effect under metabolic activation conditions and was negative for aberrations in CHO cells without metabolic activation **(MRID No. 40312701)**. This *in vitro* clastogenic activity is supported by analogue data; e.g., Acetochlor, Alachlor and Butachlor have been reported to be positive in *in vitro* cytogenetic studies.

c) In an *in vitro* unscheduled DNA synthesis (UDS) assay, Propachlor was not shown to be genotoxic in this assay up to 25 µg/mL; higher doses were cytotoxic **(MRID No. 00144512)**.

d) In an *in vivo - in vitro* rat hepatocyte UDS assay, Propachlor was not shown to be genotoxic at the concentrations (25-1000 mg/kg) tested **(MRID No. 40068401)**.

e) In a dominant lethal assay in Sprague-Dawley rats, there was no indication of a dominant lethal effect associated with dietary exposure for 10 weeks to Propachlor at dose levels up to 2500 ppm (125 mg/kg/day), an acceptably high dose **(MRID No. 43221801)**.

f) In an *in vivo* rat bone marrow cytogenetic assay, Propachlor was not shown to be clastogenic (0.05, 0.2, and 1.0 mg/kg i.p. in ethanol) in bone marrow cells of Fischer 344 rats. Cytotoxicity was not observed, and slightly higher dosing may have been appropriate **(MRID No. 00153940)**. In other studies **(Pilinskay *et al.*, 1980)**, Propachlor was positive for aberrations in mouse bone marrow, and Alachlor was positive for aberrations in rat bone marrow.

3. Structure-Activity Relationship

As shown in Figure 1, Propachlor is structurally related to Alachlor, Acetochlor, Allidochlor, Butachlor, Metolachlor and Dimethenamid.

ALACHLOR (CAS No. 15972-60-8) In accordance with the 1996 *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), Alachlor was classified as "likely" to be a human carcinogen at high doses, but "not likely" at low doses, by all routes of exposure. This conclusion was based on increased incidences of malignant and combined benign/malignant multiple tumor types in both sexes of the Long Evans rat, which occurred mainly at higher doses. Based on a consideration of modes of action for these tumors, the Cancer Peer Review Committee (CPRC) agreed that a non-linear margin of exposure (MOE) approach should be used for the purpose of risk assessment. The consensus of the CPRC was that MOEs for both the malignant mixed gastric tumors and the nasal adenomas be presented for a risk management decision.

ACETOCHLOR (CAS No. 34256-82-1) has been classified as a Group B2 Carcinogen by the HED Carcinogenicity Peer Review Committee (CPRC), CRAVE and the Science Advisory Panel (SAP), based on the evidence that administration of Acetochlor causes increased incidence of benign and malignant tumors at multiple sites in Sprague-Dawley rats (papillary adenomas of the nose/turbinates in both sexes; hepatocellular carcinomas in both sexes and thyroid follicular cell adenomas in males at excessively toxic dose levels). Also, increased incidence of benign and malignant tumors were seen at multiple sites in Swiss albino CD-1 mice (hepatocellular carcinoma in both sexes; lung carcinomas in females; uterine histiocytic sarcoma and benign ovarian tumors in females; kidney adenomas in females). Acetochlor was positive for mutations in CHO cells (with and without metabolic activation) and in mouse lymphoma cells (with metabolic activation). In addition, positive results were found for aberrations in cultured human lymphocytes and a weak positive response in an *in vivo/in vitro* UDS assay. Suggestive results were found in a *Salmonella* assay and negative results in an *in vitro* UDS assay, an *in vivo* cytogenetics test in rat bone marrow, and a mouse micronucleus test.

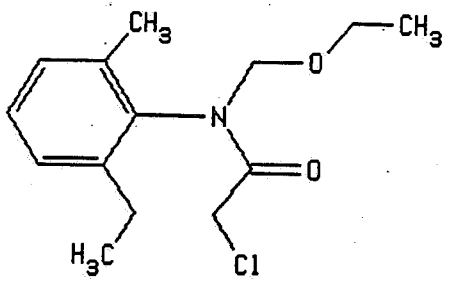
ALLIDOCHLOR (CAS No. 93-71-0) has no acceptable chronic or mutagenicity studies to support the chemical (all IBT, considered invalid) and therefore has not been evaluated by the HED Cancer Assessment Review Committee.

BUTACHLOR (CAS No. 23184-66-9) In accordance with the 1996 *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), Butachlor was classified as "likely" to be a human carcinogen by all routes of exposure. This classification is based on the poorly differenciated carcinoids (carcinomas, leiomyosarcomas and carcinosarcomas) of the stomach, adenomas/ carcinomas of the nasal mucosa, adenomas/carcinomas of the thyroid glands, and cortical tumors of the kidneys in Sprague-Dawley rats. It was weakly mutagenic in the *Salmonella* assay (usually in strain TA100), gave negative results in 4 unacceptable mammalian *in vivo* assays [bone marrow cytogenetics assay, a micronucleus test with Swiss mice, a dominant lethal assay, and a UDS assay], produced negative results in an acceptable micronucleus assay, and showed no evidence of mutagenic activity in *in vitro* gene mutation and chromosomal aberration assays with CHO cells. There is, however, evidence in the open literature (Lin et al., 1987) that Butachlor induces both chromosome aberrations and sister chromatid exchanges in CHO cells.

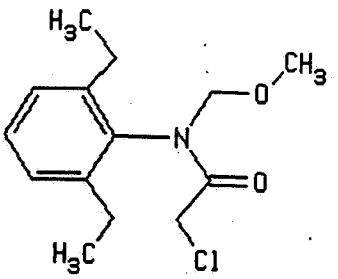
METOLACHLOR (CAS No. 51218-45-2) is classified as a Group C carcinogen and a MOE approach was recommended for estimation of human risk, based on statistically significant increases in liver adenomas and combined liver adenomas/carcinomas in Charles River female rats. The same liver neoplasia in female rats was also observed in a separate repeat study. In male rats there was a statistically significant trend, but not pair-wise significance, for liver tumors. There was no apparent increase in tumors when Metolachlor was administered in the diet to CD-1 mice in two separate studies. Metolachlor was negative in the *Salmonella* assay, mouse lymphoma gene mutation assay, and the mouse micronucleus assay. Metolachlor was also negative *in vivo* for UDS but did induce cell proliferation in hepatocytes recovered from treated rats.

DIMETHENAMID [CAS No. 87674-68-8] is classified as a Group C carcinogen, based on a chronic toxicity/carcinogenicity study in rats in which an increased incidence of benign tumors of the liver in male rats at 700 and 1500 ppm was observed. In female rats, benign tubular adenomas of the ovary were observed in increased incidence at 1500 ppm. In a 94 week dietary administration study in mice, no increase in the incidence of treated mice with benign or malignant tumors was observed. Dimethenamid was not mutagenic in the *Salmonella* assay, but induced chromosome aberrations in cultured Chinese hamster ovary cells both with and without metabolic activation. It was positive for UDS activity in primary rat hepatocytes *in vitro* at dose levels well below the cytotoxic level in one study and gave a suggestive positive response in a second UDS study. There was, however, no evidence of a genotoxic response in an *in vivo* UDS assay or a mouse micronucleus assay. In contrast, dimethenamid was shown to induce a reproducible dominant lethal effect in two independently conducted rat dominant lethal assays.

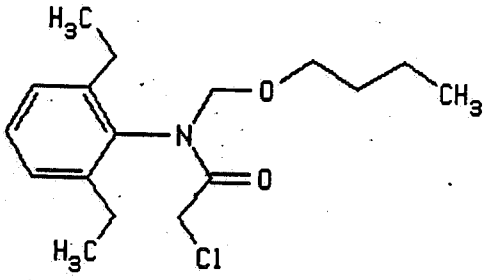
Figure 1. Compounds Structurally Related to Propachlor.



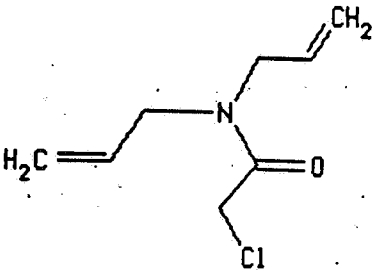
Acetochlor



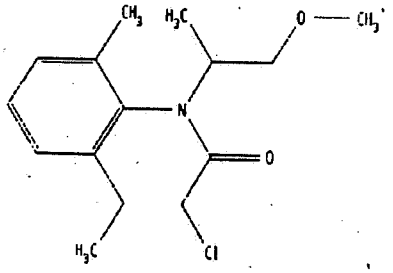
Alachlor



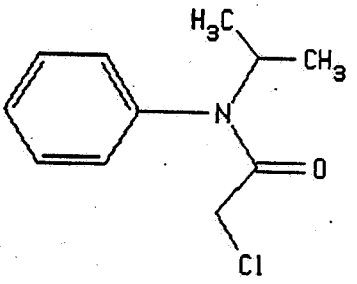
Butachlor



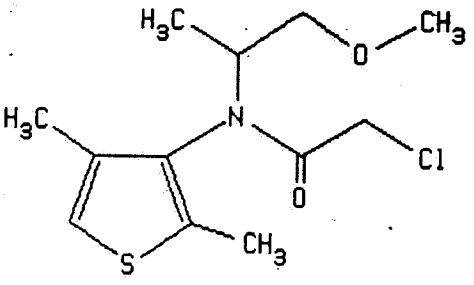
Allidochlor



Metolachlor



Propachlor



Dimethenamid

4. Acute, Subchronic, and Chronic Toxicity

A) **Acute Toxicity**

Propachlor is not acutely toxic *via* the oral, dermal or inhalation routes of exposure (Toxicity Categories III, IV, III, respectively), is not skin irritant (Toxicity Category IV), but is a severe eye irritant (Toxicity. Category I). In an acute neurotoxicity study in rats, the NOEL was 175 mg/kg and the LOEL, 350 mg/kg/day, was based on an increase in landing foot splay at the peak effect time of 7 hours.

B) **Subchronic Toxicity**

In a subchronic feeding study, **(MRID No. 00152151)**, Sprague-Dawley rats, (30/sex/dose) were administered Propachlor (96.1%) in the diet at dose levels of 0, 300, 1500, or 7500 ppm (15, 75 or 375 mg/kg/day, respectively) for 90 days. There were no deaths. During the first month only, hyperactivity was displayed by the high-dose rats. There was a dose-related decrease in body weight throughout the study (terminal sacrifice: males 90% and 39% of control/females 92% and 63% of control for the mid- and high-dose, respectively). There was a negative body-weight gain during the first week in both sexes at the high-dose level, and overall (males 13% of control/females 31% of control). In both sexes at 7500 ppm, food consumption was decreased during the first 4-5 weeks. Effects (increase in cholesterol and, decreases in glucose, protein, as well as organ weights) observed at the high dose were attributed to poor nutrition, due to the poor palatability of the test material and not to a toxic effect. No adverse effects were observed at the mid- and low-dose levels in either sex. The NOEL was 1500 ppm (75 mg/kg/day). This study was classified as unacceptable due to the lack of effects at palatable dose levels and the lack of pair fed controls for comparison to dose levels that were not palatable.

In a subchronic feeding study **(MRID No. 00256450)**, Crl:CD®-1 (ICR)BR mice (30/sex /dose) received Propachlor (96.1%) in their diet at dose levels of 0, 500, 1500 or 5000 ppm (75, 225 or 750 mg/kg/day, respectively) for 90 days. There were no deaths. There was a dose-related decrease in body weight of the males throughout the study (week 13: 95.6% and 91.1% of control for the mid- and high-dose, respectively). In females, there was a dose-related decrease in body weight during the first 6 weeks (week 6: 93.3% and 91.6% of control at the mid- and high-dose levels, respectively), but during the last half of the study, the mid-dose females displayed the lowest body weight compared to the control (week 13: 92.2% and 95.8% of control at the mid- and high-dose levels, respectively). Food consumption was decreased mainly in females at the high dose. There was a dose-related decrease in leukocytes in both sexes at week 7, and a decrease

was observed in the mid- and high-dose males at study termination. There was a dose-related decrease in kidney weight in males that was statistically significant at all doses and relative kidney weight was significantly decreased in the high-dose males. Liver weight was increased in the mid- and high-dose males and in the high-dose females. There was a dose-related increase in relative liver weights in both sexes. Centrilobular hepatocyte enlargement (0, 2, 8, or 19 of 20 males in the control, low, mid-and high-dose, respectively, and in 3 of 20 high-dose females) was observed at all dose levels in the males and in the high-dose females. A NOEL was not established. This study was classified as unacceptable because a NOEL could not be established due to question regarding liver effects raised during a study audit which were not addressed by the study author.

c) Chronic Toxicity

In a combined chronic toxicity/carcinogenicity study (discussed in Section III.1, **MRID 44168301**), F344 rats received Propachlor (97.83% a.i) in their diet at 0, 100, 300, 1000, or 2500 (males)/ 5000 (females) ppm for 24 months. There were no adverse effects on survival or clinical signs in either sex. Both sexes at the highest dose level displayed decreased body weight throughout most of the study (♂ 93%/♀ 72% of control value at study termination), which was accompanied by a decreased food intake (attributable to poor palatability of the test material). Small decreases in body weight (♂ 96-97%/♀ 93-97% of control) and food consumption were observed in both sexes at the 1000 ppm dose level also. Body-weight gains at the two highest dose levels of both sexes were significantly decreased throughout much of the study, with the deficit for the first 3-month interval being 93%/89% of the control for males/females at 1000 ppm and 82%/79% of the control for males/females at the highest dose level, respectively. Several clinical pathology findings (initial decrease in red cell indices suggesting a mild anemia, increases in platelets/white blood counts in females, decreases in serum enzymes, increased GGT levels) may be treatment-related, although wide variability occurred in both sexes. At both the 12-month and terminal sacrifices, increased liver weight (absolute and relative-to-brain) was observed in females at the highest dose, but males at the highest dose level displayed increased liver weight only at the interim sacrifice. At the highest dose level, kidney weights (absolute and relative-to-brain) were decreased in both sexes at the terminal sacrifice. At study termination, increased testicular weight was observed in males at the highest dose level and decreased thyroid weight was observed in females at the highest dose level. In the stomach, herniated mucosal glands (submucosa/tunica muscularis), mucosal hyperplasia of the pylorus, and pyloric cyst(s) were observed only in treated rats of both sexes, and the incidence and severity increased with dose in males. Females at 1000 ppm dose and both sexes at the high dose also displayed erosion/ ulceration of the glandular mucosa of the stomach.The incidence and severity of hepatocellular hypertrophy (centrilobular/midzonal) were increased in a dose-

related manner in both sexes.. Dosing was considered adequate, based on the decrease in food consumption and a concomitant decrease in body-weight gain. The LOEL was 300 ppm (♂ 16.1/♀ 19.3 mg/kg/day), based on stomach lesions in males and liver lesions in both sexes and the NOEL was 100 ppm (♂ 5.4/♀ 6.4 mg/kg/day).

In a 2-year chronic toxicity/carcinogenicity study (discussed in Section III.2, **MRID No.40473101**), Sprague-Dawley rats were fed diets containing Propachlor (96.1%) at 0, 10, 50, or 500 ppm for 104 weeks. There was no adverse effect on survival of either sex, and the most common cause of death was listed as pituitary neoplasia. Males displayed comparable body weights and body-weight gains among the groups throughout the study, and females displayed a slight (95-99% of control) decrease in body weight at the mid- and high-dose levels compared to the control at several times during the study. Thyroid weight (absolute and relative to body and brain) was increased in high-males at the interim sacrifice only, and there were no organ weight differences observed in either sex at study termination. A NOEL was not established. The high dose tested was not adequate to assess either the chronic toxicity or carcinogenicity of Propachlor.

In a carcinogenicity study (discussed in Section III.3, **MRID No 44069801**) CD-1 mice received Propachlor (97.83% a.i.) in the diet at 0, 100, 500, 1500, or 6000 ppm for 18 months. There were no adverse effects on survival or clinical signs in either sex. Decreased body weight (♂ 89%/♀ 86% of control value at study termination) and body-weight gains (overall gain ♂ 72%/♀ 68% of control) were observed in males at 1500 ppm and in both sexes at the highest dose level throughout most of the study following the ramping phase of the dosing procedure. These decreases were accompanied by decreases in food consumption. This latter effect can be attributed to the known poor palatability of Propachlor. Comparable increases in platelet counts were observed in both sexes at the highest dose level compared to the controls. At both the 12-month and terminal sacrifices in both sexes, there was a dose-related increase in liver weight. At the highest dose, kidney weights (absolute and relative-to-brain) were decreased in males at both the 12-month and terminal sacrifices and in females at the terminal sacrifice. In the stomach, herniated mucosal glands in the submucosa/tunica muscularis were observed in both sexes at the highest dose and in some males at the next highest dose. Males at the highest dose level also displayed erosion/ulceration of the glandular mucosa of the stomach. Several non-neoplastic lesions [hepatocellular hypertrophy (centrilobular/ midzonal), necrosis of individual hepatocytes, eosinophilic foci, telangiectasis, and pigment deposition in Kupffer cells in the males and hepatocellular hypertrophy (periportal), mononuclear cell infiltrate, and pigment deposition in Kupffer cells in the females] indicative of liver toxicity were observed in both sexes at the highest dose level and in males at the next highest dose. The LOEL was 500 ppm (75 mg/kg/day and 100 mg/kg/day in males and females, respectively), based on increased liver weights and microscopic lesions in the liver. The NOEL was 100 ppm (14.6 mg/kg/day and 19.3 mg/kg/day in males and females, respectively).

In a 78-week study(**MRID No.40162501**) in CD-1 mice (50/sex/dose), Propachlor was administered via the diet at dose levels of 0, 10, 50, and 500 ppm. There was no adverse effect on survival. Survival was comparable among the males, but due to the unusually high survival rate in the female control group, all treated female groups displayed a lower survival rate compared to the control (not dose-related). Body weight and food consumption were comparable among the groups for both sexes. There was an increase in the incidence of a hunched and thin appearance in the mid- and high-dose mice of both sexes. There was no increase in any tumor type with treatment in either sex. The high dose used in this study was the NOEL determined in a 90-day study. In the 90-day study, when tested at 500, 1500, or 5000 ppm, treatment-related effects included: (i) decreased body weight in both sexes at the mid- and high-dose levels (♂ 95.6% and 91.1% of control, respectively and ♀ 92.2% and 95.8% of control, respectively); (ii) liver lesions (centrilobular hypertrophy in 0, 2, 8, and 19 of 20 males/group and in 3 of 20 high-dose females); and (iii) increased liver weights (in mid-dose males and in both sexes at the high dose). The dose levels tested in the carcinogenicity study were not adequate to assess the carcinogenicity of Propachlor.

V. WEIGHT OF EVIDENCE CONSIDERATIONS

The Cancer Assessment Review Committee was asked to consider the following Weight-of-the-Evidence in evaluating the carcinogenic potential of Propachlor:

1. Combined Chronic Toxicity and Carcinogenicity Study in F-344 Rats.

In Fischer-344 rats fed 0, 100, 300, 1000 or 2500/5000 ppm for 24 months, there was no adverse effect on survival of either sex. Non-neoplastic lesions observed only in treated rats of both sexes included herniated mucosal glands (submucosa/tunica muscularis), mucosal hyperplasia of the pylorus, and pyloric cyst(s). The incidence and severity increased with dose in the males.

The only neoplastic finding was the presence of a carcinoma in the glandular stomach in one male rat at 2500 ppm; an uncommon tumor in rats. No stomach tumors were seen in historical control rats (250 males and 250 females) at the testing laboratory . No stomach tumors were seen in female rats in this study.

2. Combined Chronic Toxicity and Carcinogenicity Study in Sprague-Dawley Rats.

In Sprague-Dawley rats fed diets containing Propachlor at dose levels of 0, 10, 50, or 500 ppm for 105 weeks, there were no adverse effects on survival of either sex. Neoplastic findings were seen in the thyroid of both sexes and in the ovary of the females.

Male rats had significant increasing trends in thyroid C-cell adenomas and combined adenomas/carcinomas, and there was a significant difference in the pair-wise comparison of the 500 ppm dose group with the controls for the combined adenomas/carcinomas.

Female rats had significant positive trends in thyroid C-cell adenomas and combined adenomas/carcinomas and in ovarian granulosa/theca cell benign tumors and combined benign/malignant tumors. There was a significant difference in the pair-wise comparison of the 500 ppm group with the controls for the combined benign/malignant ovarian granulosa/theca cell tumors. Historical control data from the testing laboratory were not available for either tumor type.

3 Carcinogenicity Study in CD-1 Mice

In CD Mice fed Propachlor at dose levels of 0, 100, 500, 1500, or 6000 ppm for 18 months, there was no adverse effect on survival of either sex. Neoplastic findings were observed in the liver of the males compared with the controls.

Male mice had significant increasing trends and significant differences in the pair-wise comparisons at 6000 ppm when compared to controls, for hepatocellular adenomas and adenomas and/or carcinomas combined. There was a significant trend in hepatocellular carcinomas also. No significant increase in tumor incidence was seen in males at doses below 6000 ppm . The tumor incidences exceeded the historical control incidence. No liver tumors were seen in female mice.

4. Mutagenicity From the submitted studies, Propachlor was positive in the CHO/HGPRT assay and in the CHO/chromosomal aberration assay under metabolic conditions, Propachlor was negative in the *in vitro* UDS assay, the *in vivo - in vitro* rat hepatocyte DNA repair assay, and the dominant lethal assay. A published report indicates that Propachlor is positive for aberrations in mouse bone marrow (Pilinskaya et al., 1980). The data showing *in vitro* clastogenic activity for Propachlor compare favorably with the findings from similar testing with other compounds in this chemical class; i.e., Alachlor, Acetochlor, and Dimethenamid.

5. Structure-Activity Relationship.

Alachlor is classified as a "likely" human carcinogen at high doses but "not likely" at low doses (malignant and combined benign/malignant multiple tumor types mainly at high doses in both sexes of Long-Evans rats).

Acetochlor is classified as a Group B2 carcinogen based on benign and malignant tumors observed at multiple sites in Sprague-Dawley rats (papillary adenomas of nose/turbinates in both sexes, hepatocellular carcinomas in both sexes, and thyroid follicular cell adenomas in males at excessive doses) as well as benign and malignant tumors seen at multiple sites in CD-1 mice (hepatocellular carcinoma in both sexes, lung carcinomas in females, uterine histiocytic sarcoma and benign ovarian tumors in females, kidney adenomas in females).

Butachlor is classified as a "likely" human carcinogen based on the tumors of the stomach (females), nasal mucosa and thyroid glands (both sexes), and kidneys (males) of Sprague-Dawley rats. In mice Butachlor induced alveolar/bronchiolar adenomas of the lung at doses that were considered to be excessive to assess carcinogenicity.

Metolachlor is classified as a Group C carcinogen based on hepatocellular adenomas and combined hepatocellular adenomas/carcinomas is seen in female Charles River rats in two studies; only significant trend for hepatocellular tumors were seen in male rats. No evidence of carcinogenicity was seen in CD-1 mice.

Dimethenamid is classified as a Group C carcinogen (benign liver tumors in male rats, benign tubular adenomas of the ovary in female rats; no apparent increase in tumors in mice).

VI. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

The Committee's assessment of the weight-of-the-evidence is presented below:

1. Carcinogenicity

Evidence for carcinogenicity was seen in the stomach of male Fischer 344 rats, the thyroid glands of male and the ovaries of female Sprague-Dawley rats, as well as the liver of male CD-1 mice.

(i) Stomach

In Fischer-344 rats, a single carcinoma of the glandular stomach was seen in one male at the high dose (2500 ppm or 125.3 mg/kg/day). This tumor was attributed to treatment since:

- stomach tumors are rare in rats.
- the incidences and severity of the non-neoplastic lesions (herniated mucosal glands, mucosal hyperplasia of the pylorus and pyloric cysts) observed only in treated rats both sexes increased with dose in males.
- no stomach tumors were observed in historical control rats at the testing laboratory.
- stomach tumors were also seen in rats treated with structural analogs (Alachlor and Butachlor).

In addition, the stomach tumor seen in this study was of a different cell type from the stomach tumors induced by the analogs. Alachlor induced osteosarcomas, malignant mixed gastric tumors and combined gastric adenocarcinomas and/or malignant mixed gastric tumors in both sexes of Long-Evans rats. Butachlor induced carcinomas, leiomyosarcomas and carcinosarcomas of the glandular stomach in female Sprague-Dawley rats. It was noted that the stomach tumor seen in this study arose from the pyloric regions of the stomach whereas stomach tumors seen with the other chloracetanilides occurred in the fundic region of the stomach in Sprague-Dawley rats. In depth analysis of the Butachlor-induced stomach tumors indicates that most, if not all of the tumors appear to be poorly differentiated carcinoids of the fundus, neoplasms of enterochromaffin-like (ECL) cells that normally produced histemine. The Committee concluded that this tumor type was significant and of concern. No data were provided for a mode of action for induction of this tumor. It was concluded that the dose levels tested in this study were adequate to assess the carcinogenic potential of Propachlor.

(ii) **Thyroids**

In the Sprague-Dawley rats, C-cell adenomas, carcinomas and combined adenomas/ carcinomas of the thyroid glands were observed in both sexes of rats including the controls. The thyroid tumors were attributed to treatment for the following reasons:

- there were significant positive trends for adenomas (males, p=0.039; females, p= 0.048) and combined adenomas/carcinomas (males, p=0.012; females, p=0.037).
- the incidence of combined adenomas/carcinomas in males (6/47, 13%) at the high dose (23.88 mg/kg/day) was significantly (p= 0.047) increased when compared to controls (1/50, 2%).
- the incidences in females at the high dose (5/47, 11%), although not statistically significant, was higher than the controls (3/45, 7%).

The Committee postulated that since the highest dose level tested was not adequate, a higher dose might have resulted in elevated tumor incidences. In addition, thyroid follicular cell tumors were seen with structural analogs (Acetochlor, Alachlor and Butachlor). Historical control data from the testing laboratory were not available.

(iii) **Ovaries**

The ovarian granulosa/theca cell benign and combined benign/malignant tumors seen in female Sprague-Dawley rats were attributed to treatment because:

- there were significant positive trends for both the benign (p=0.005) and combined benign/malignant (p=0.002) tumors.
- there was significance (p=0.033) in pair-wise test for the incidence of combined benign/malignant tumors at the high dose when compared to controls;
- the incidences at the high dose (23.88 mg/kg/day) were higher for all three types [4/47 (9%), benign; 1/47 (2%) malignant; and 5/47 combined benign/malignant (11%] when compared to control (0/44, 0%).
- the incidences exceed the historical control range of 0 - 5%.

The Committee concluded that the thyroid and ovarian tumors occurred at doses that were less than adequate to assess the carcinogenic potential of Propachlor.

(iv) Liver

In CD-1 mice, the hepatocellular tumors seen in males at the high dose (847.3 mg/kg/day) were attributed to treatment because:

- there were significant positive trends for all three types (adenomas, p=0.000, carcinomas, p=0.002 and combined adenomas/carcinomas, p=0.000).
- the incidences of adenomas (29/40, 72.5%) and combined adenomas/carcinomas (31/49, 63%) were significantly (p=0.000) increased when compared to controls (0/49, 0%).
- the incidences exceeded the historical control range for adenomas (0-21.7%) as well as combined adenomas/carcinomas (5.2 - 20.3%).

It was concluded that the dose levels tested (almost the Limit-Dose) were adequate to assess the carcinogenicity of Propachlor.

2. Mutagenicity

The *in vitro* clastogenic activity of Propachlor is consistent with the results obtained in similar testing with structural analogs (Acetochlor and Dimethenamid).

3. Structure Activity Relationship

In rats, Propachlor induced tumors at the same sites (stomach and thyroids) as Acetochlor (thyroids), Alachlor (stomach/thyroids), and Butachlor (stomach/thyroids) but it did not induce either liver tumors seen with Metolachlor and Dimethenamid or nasal tumors seen with Acetochlor, Alachlor and Butachlor. In mice, the hepatocellular tumors seen with Propachlor were seen with Acetochlor.

VII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified Propachlor as a **"likely"**human carcinogen. The weight-of-the-evidence for this classification are as follows:

(i) multiple tumors at multiple sites including rare stomach tumor in male Fischer 344 rats, thyroid tumors in male and ovarian granulosa/theca cell tumors in female Sprague-Dawley rats and hepatocellular tumors in male CD-1 mice;

(ii) i*n vitro* clastogenic activity;

(iii) tumors seen at one or more of the same sites with three structurally related chloracetanilide compounds;

(iv) lack of data on mode of actions; and

(v) the relevance of the observed tumors to human exposure.

VIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose approach for human risk characterization and extrapolation should be based on both the neoplastic (ovarian tumors in female rats and liver tumors in male mice) and non-neoplastic (liver hypertrophy in mice) lesions. The "points of departure" for extrapolations are 50 ppm (2.4 mg/kg/day) for neoplastic lesions (ovarian tumors) and 500 ppm (75 mg/kg/day) for non-neoplastic lesions (liver hypertrophy).

IX BIBLIOGRAPHY

MRID No.	CITATION
00152151	Three Month Feeding Study of Propachlor to Albino Rats [Project No. 83-083, Study No. 83007; dated 11/19/84;]; HED Document No. 008277.
00153939	CHO/HGPRT Gene Mutation Assay with Propachlor. Final Report No. EHL 840083.
00153940	Assessment of the Mutagenic Potential of Propachlor Utilizing the Acute In Vivo Rat Bone Marrow Cytogenetics Assay. SRI Project No. LSC-7405 (SR-84-180).
00257647	Evaluation of the Potential of Propachlor to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures. Project No.LSC-7538; Monsanto Study No. SR-84-239.
00256450	Three Month Feeding Study of Propachlor to Albino Mice. [Study No. 820061; Project No. ML-81-72; dated 10/9/84;; HED Document No. 006677.
00157496	Lamoureux, G.; Davison, K. (1975). Mercapturic Acid Formation in the Metabolism of Propachlor, CDAA, and Flourodifen in the Rat. Published study in Pesticide Biochemistry and Physiology 5: 497-506.
00157497	Bakke, J.; Feil, V.; Price, C. (1976). Replacement of a Chlorine with a Methylsulfonyl Group in the Metabolism of Propachlor (2-chloro-N-isopropylacetanilide). Published study in Biomedical Mass Spectrometry 3: 266-229.
00157498	Bakke, J.; Price, C. (1979). Metabolism of 2-chloro-N-isopropyl-acetanilide (Propachlor) in the Rat. Published study in J. Environ. Sci. Health B14(4): 427-441.
00157499	Larsen, G.; Bakke, J. (1979). Studies on the Origin of the Methylsulfonyl-Containing Metabolites from Propachlor. Published study in J. Environ. Sci. Health B14(5): 495-504
00157500	Pekas, J.; Larsen, G.; Feil, V. (1979). Propachlor Detoxication in the Small Intestine: Cysteine Conjugation. Published study in the Journal of

Toxicology and Environmental Health 5: 653-662.

00157502 Larsen, G.; Bakke, J. (1981). Enterohepatic Circulation in Formation of Propachlor (2-chloro-N-isopropylacetanilide) Metabolites in the Rat. Published study in Xenobiotica 11(7): 473-480.

00157503 Rafter, J.; Gustafsson, J.; Bakke, J.; et al. (1983). Studies on the Re-establishment of the Intestinal Microflora in Germ-Free Rats with Special Reference to the Metabolism of N-isopropyl-[alpha]-chloroacetanilide (Propachlor). Xenobiotica 13(3): 171-178.

00157504 Bakke, J. Price, C. (1979). The metabolic Fate of N-isopropyl-N-phenyloxamic Acid in the Rat and the Milk Goat. J. Environ. Sci. Health B14(3): 279-290..

00157505 Larsen, G.; Bakke, J. (1983). Metabolism of Mercapturic Acid-Pathway Metabolites of 2-Chloro-isopropylacetanilide (Propachlor) by Gastrointestinal Bacteria. Xenobiotica 13(2): 115-126.

00157506 Bakke, J. Rafter, J. Larsen, G.; et al. (1981). Enterohepatic Circulation of the Mercapturic Acid and Cysteine Conjugates of Propachlor. Drug Metabolism and Disposition 9(6): 525-528.

00157507 Bakke, J. Rafter, J. Larsen, G.; et al. (1981). Enterohepatic Circulation of the Mercapturic Acid and Cysteine Conjugates of Propachlor. Drug Metabolism and Disposition 9(6): 525-528.

40068401 Evaluation of the Potential of Propachlor to Induce Unscheduled DNA Synthesis in the in vivo--in vitro Rat Hepatocyte DNA Repair Assay. Project No.LSC-2021; Monsanto Study No. SR-86-85.

40162501 Oncogenicity Study in Mice. HL-83-349. Project No. 241-159. January 19, 1987.

40312701 In vitro Cytogenetics Study of Propachlor. Project No. EHL 86047; R.D. No. 807.

40473101 Combined Chronic Toxicity and Oncogenicity Study of Propachlor in Rats (Propachlor). (1987). Study No. HL-83-350/241-160, dated 8/14/87.

43221801 Dominant Lethal Study of Propachlor in Sprague-Dawley Rats. Project No. ML-93-145: EHL 93050: MSL-1355.

44069801 Oncogenicity Study of Propachlor Administered in Feed to CD-1® Mice for 18 Months. Monsanto Study No. ML-93-191; Project No. EHL 93076, dated 7/19/96;; HED Document No. 012097.

44168301 Combined Chronic/Oncogenicity Study of Propachlor Administered in Feed to F-344 Rats for 24 Months (1996). Monsanto Study No. ML-93-190, Laboratory Project No. EHL 93075, MSL No. 14861, RD 1363. dated 11/26/96.

---------- Lin, M.F. Wu, C.L, Wang, T.C. (1987). Pesticide Clastogenicity in Chinese hamster ovary cells. Mutat.Res. 88:241-250