*C*ANCER *A*SSESSMENT *D*OCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

DICLOFOP-METHYL
(SECOND REVIEW)
P.C. CODE: 110902

FINAL REPORT

24-MAY-2000

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION:

_____
Robert Fricke, Toxicologist

DOCUMENT PREPARATION:

_____
Sanjivani Diwan, Executive Secretary

<u>CARC/MTARC MEMBERS IN ATTENDANCE:</u>   (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke   _____

William Burnam   _____

Ghazi Dannan   _____

Kerry Dearfield   _____

Vicki Dellarco   _____

Virginia Dobozy   _____

Karen Hammernick   _____

Yiannakis Ioannou   _____

Nancy McCarroll   _____

Esther Rinde   _____

Joycelyn Stewart   _____

Clark Swentzel   _____

Linda Taylor   _____

Pauline Wagner   _____

 Yin-Tak Woo   _____

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

John Pletcher,   Pathology   _____

Lori Brunsman,   Statistical Analysis   _____

<u>OTHER ATTENDEES</u>

Penny Fenner-Crisp/OPP

_____

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EXECUTIVE SUMMARY

Diclofop-methyl also known as Hoelon was initially reviewed by the CPRC (Cancer Peer Review Committee) of the Health Effects Division of the Office of Pesticide Programs on February 10, 1993. At this meeting, a carcinogenicity study in the mouse and a combined chronic feeding/carcinogenicity study in the rat were evaluated. The mouse study was acceptable while the carcinogenicity portion of the rat study was found to be unacceptable (MTD not achieved). Diclofop-methyl was determined by the CPRC to be negative in both *in vitro* and *in vivo* mutagenicity assays. The CPRC classified diclofop-methyl as a possible human carcinogen (Group C) and recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on a statistically significant increase in combined hepatocellular adenomas/carcinomas in male and female B6C3F1 mice (CPRC, 1993), and requested a new rat study.

On January 5, 2000, the Cancer Assessment Review Committee (CARC) met to reconsider the carcinogenicity classification of diclofop-methyl in light of the newly submitted rat study. Additionally, on March 29, 2000, a joint meeting of the Health Effects Division's Mechanism of Toxicity Assessment Review Committee (MTARC) and CARC was held to discuss the mode of action of diclofop-methyl in liver carcinogenesis. The CARC concluded that diclofop-methyl was carcinogenic in male and female Wistar rats because there were significant (p<0.01) increases (both pair-wise and trend) in the 450 ppm group for hepatocellular adenomas and carcinomas in males as well as combined adenomas/carcinomas in both sexes. The incidence of adenomas and carcinomas exceeded the historical control range. The CARC considered these tumors to be treatment-related. In addition, a significantly increasing trend was noted for testicular Leydig cell tumors (p<0.05) in males and the occurrence of these tumors was preceded by hyperplasia of Leydig cells. In females, a significant (p<0.05) increasing trend was noted for uterine glandular polyps and thyroid follicular cell adenomas. The Committee considered these tumors to be supportive of the carcinogenic potential of diclofop-methyl. The dosing at 450 ppm was considered to be adequate and not excessive based on the decrease in body weight gain, and histopathological changes in the liver in both male and female rats. The CARC also reaffirmed the CPRC's decision that diclofop-methyl was a hepatocarcinogen in both sexes of mice and was not mutagenic in both *in vitro* and *in vivo* assays. Structurally related diphenyl ethers cause liver tumors in rats and/or mice. The MTARC and CARC agreed that there is some evidence of peroxisome proliferation in the submitted studies, however, they lack the depth and quality to establish peroxisome proliferation as the mode of action for nonmutagenic hepatocarcinogenicity of diclofop-methyl.

According to EPA's Draft Cancer Risk Assessment Guidelines (EPA, 1999), the CARC classified diclofop-methyl as **"likely to be carcinogenic to humans "** by the oral route based on the occurrence of both benign and malignant hepatocellular tumors in both sexes of rats and mice. The CARC recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on the most potent of the tumors observed in mice. This approach is supported by the lack of confirmation of the mode of action.

I. INTRODUCTION

Diclofop-methyl also known as Hoelon (P.C .Code: 110902) was initially reviewed by the CPRC on February 10, 1993. At this meeting, a carcinogenicity study in the mouse and a combined chronic feeding/carcinogenicity study in the rat were evaluated. The mouse study was acceptable while the carcinogenicity portion of the rat study was found to be unacceptable because an MTD was not achieved. The CPRC classified diclofop-methyl as a possible human carcinogen (Group C). The Committee recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on a statistically significant increase in combined hepatocellular adenomas/carcinomas in male and female B6C3F1 mice of both sexes.

On January 5, 2000, the Cancer Assessment Review Committee [CARC] met to discuss the newly submitted rat study and reclassify diclofop-methyl under the Agency's *Draft Carcinogen Risk Assessment Guidelines* (July, 1999).   Additional data/information previously not available to the CPRC were presented by Dr. Robert Fricke, which included a rat chronic/carcinogenicity study, a revised statistical analysis of the rat tumor data, structure-activity of related compounds as well as a proposed mode of action.

II. EVALUATION OF CARCINOGENICITY STUDIES

Conclusions of the 1993 CPRC Meeting Regarding the Mouse Study

In a 102-week combined chronic toxicity/carcinogenicity study in HOE:NMRKf (SPF71) male and female mice (260 mice/sex/group in the controls group and 130 mice/sex/dose groups), there was a significant (P<0.01 or 0.05) increase in the pair-wise comparisons of the 20 ppm dose group with controls for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. The incidences in male mice were as follows: adenomas: 20/113, 18% vs 5/230, 2% in controls; carcinomas: 12/85, 14% vs 3/184, 2% in controls; combined adenomas/carcinomas: 32/113, 28% vs 8/230, 3% in controls. Among females, the incidences were: adenomas: 7/112, 6% vs 0/208, 0% in controls; carcinomas: 3/112, 3% vs 0/208, 0% in controls; combined: 10/112, 9% vs 0/208, 0% in controls.   Statistically significant trends were evident in both sexes for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. The incidences of these tumors exceeded the historical control ranges provided by the Registrant. The CPRC concluded that liver tumors in male and female mice were treatment-related. The mouse data are discussed in detail in the CPRC (1993) document on diclofop-methyl [HED Document No. 012870].

New Rat Data Presented to The CARC

Prior to the January 5, 2000 meeting of the CARC, the Registrant submitted a new chronic toxicity/carcinogenicity study in Wistar rats. The study was submitted in response to CPRC's recommendation to fulfill the guideline requirement. The new data were evaluated by the Committee and are discussed below.

A. Combined Chronic Toxicity/Carcinogenicity Study with Diclofop-Methyl in Wistar Rats

Citation: Ehling, G., and H. Donaubauer (1996) Combined chronic toxicity (12 and 24 months) and carcinogenicity (24 months) study in rats. Hoechst Aktiengesellschaft, D-65926 Frankfurt am Main. Laboratory Report Number 92.0475. January 8, 1996, 43927302, Unpublished

Experimental Design: Wistar (Hoe:WISKf(SPF71)) rats (50/sex/dose) were dosed at dietary levels of 0, 4.5, 45, or 450 ppm (0, 0.22, 2.2 or 22.5 mg/kg/day, respectively) of diclofop-methyl for 104 weeks of the carcinogenicity study (MRID 43927302). An additional 20 rats per sex per dose were designated for the 104-week toxicity study, and another ten rats per sex per dose were designated for interim sacrifice at week 53. Female rats were also dosed at 900 ppm in the carcinogenicity study, but were sacrificed at week 24 when this dose proved excessive. The 900 ppm females are not included in this analysis.

B. Discussion of Tumor Data and Comparison with Historical Control Data

Male rats had significant differences in the pair-wise comparisons of the 450 ppm dose group with the controls, for hepatocellular adenomas, carcinomas, and adenomas/carcinomas combined, all at $p < 0.01$ with significant increasing trends (Table 1). The tumor incidences were as follows: adenomas: 14/69, 20% vs 0/70, 0% in controls; carcinomas: 18/69, 26% vs 0/70, 0% in controls; combined: 29/69, 42% vs 0/70, 0% in control). The liver tumors were considered by the CARC to be treatment-related. There was also a significant increasing trend in testicular Leydig cell tumors at $p < 0.05$. The incidences were 6%, 19% and 21% at 4.5, 45 and 450 ppm, respectively vs 11% in controls (Table 2) . The occurrence of testicular tumors was preceded by hyperplasia of Leydig cells in the testes. The CARC therefore, determined that this finding was possibly treatment-related.

Female rats had significant differences in the pair-wise comparisons of the 450 ppm dose group with the controls, for hepatocellular carcinomas, and adenomas/ carcinomas combined, all at $p < 0.01$ with significant increasing trends (Table 3).   The increased incidence of combined adenomas/carcinomas was driven by the increase in carcinomas. The tumor incidences were as follows: carcinomas: 14/70, 20% vs 1/70, 1% in controls; combined: 19/70, 27% vs 2/70, 3% in controls. The increased incidence of adenomas at 450 ppm (6/70, 9% vs 1/70, 1% in controls) was of borderline significance (p=0.058). There was also a significant increasing trend in hepatocellular adenomas at $p < 0.01$. There were significant increasing trends (p<0.05) in uterine glandular polyps (3%, 1% and 6% at 4.5, 45 and 450 ppm, respectively vs 0% in controls) and thyroid gland follicular cell adenomas 0%, 7% and 7% at 4.5, 45 and 450 ppm, respectively vs 1% in controls)(Table 4). The CARC considered that the increased incidences of uterine glandular polyps and thyroid follicular cell adenomas were supportive of carcinogenic potential of diclofop-methyl and therefore, could not be discounted.

Historical control data are presented in Tables 5 and 6. Table 5 is the historical control data for Pharma Development Corporate Toxicology of Hoechst and represents the tumor data for a limited number of studies (three). Since some of the tumors were not observed in the Pharma data base, the historical control data for Registry of Industrial Toxicology Animal Data (RITA) are included (Table 6); the RITA tumor data base (of which the Pharma data is a part) was compiled from 24 studies conducted in Germany by different companies.

The incidence of liver adenomas and carcinomas in males and females, and thyroid follicular cell adenomas in females, exceeded the Pharma and/or RITA historical control ranges at the 450 ppm dose level. Leydig cell tumors, uterine glandular polyps, and adrenal medulla adenomas (males) exceeded the Pharma historical control data, but were within that of RITA.

Table 1. Diclofop-Methyl - Wistar (Hoe:WISKf(SPF71)) Rat Study
Male Liver Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p values) (Brunsman, 1999)

	Dose (ppm)			
	0	4.5	45	450
Adenomas (%)	0/70 (0)	1/69 (1)	0/69 (0)	14[a]/69 (20)
p=	0.000**	0.496	1.000	0.000**
Carcinomas (%)	0/70 (0)	0/69 (0)	0/69 (0)	18[b]/69 (26)
p=	0.000**	1.000	1.000	0.000**
Combined (%)	0/70 (0)	1/69 (1)	0/69 (0)	29[c]/69 (42)
p=	0.000**	0.496	1.000	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
[a]First adenoma observed at week 103, dose 450 ppm.
[b]First carcinoma observed at week 88, dose 450 ppm.
[c]Three animals in the 450 ppm dose group had both an adenoma and a carcinoma.
Note: There were no liver adenomas or carcinomas in any interim sacrifice animals.
        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 2. Diclofop-Methyl - Wistar (Hoe:WISKf(SPF71)) Study

Male Adrenal Gland and Testes Tumor Rates[+] and Exact
Trend Test and Fisher's Exact Test Results (p values) (Brunsman, 1999)

	Dose (ppm)			
	0	4.5	45	450
Adrenal Gland Medulla Adenomas (%)	1/70 (1)	2/69 (3)	1/69 (1)	4[a]/70 (6)
p=	0.076	0.495	0.748	0.183
Testes Leydig Cell Tumors (%)	8/70 (11)	4/69 (6)	13/69 (19)	15[b]/70 (21)
p=	0.020*	0.190[n]	0.163	0.085

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
[a]First adrenal gland medulla adenoma observed at week 103, dose 450 ppm.
[b]First testes Leydig cell tumor observed at week 96, dose 450 ppm.
[n]Negative change from control.

Note: There were no adrenal gland medulla adenomas or testes Leydig cell tumors in any interim sacrifice animals.
Significance of trend denoted at <u>control</u>.
Significance of pair-wise comparison with control denoted at <u>dose</u> level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Diclofop-Methyl - Wistar (Hoe:WISKf(SPF71)) Rat Study

<u>Female</u> Liver Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p values) (Brunsman, 1999)

	Dose (ppm)			
	0	4.5	45	450
Adenomas[a]	1/70	0/69	1/69	6/70
(%)	(1)	(0)	(1)	(9)
p=	0.002**	0.504[n]	0.748	0.058
Carcinomas	1/70	0/69	3/69	14[b]/70
(%)	(1)	(0)	(4)	(20)
p=	0.000**	0.504[n]	0.304	0.000**
Combined	2/70	0/69	4/69	19[c]/70
(%)	(3)	(0)	(6)	(27)
p=	0.000**	0.252[n]	0.334	0.000**

[+] Number of tumor-bearing animals/Number of animals examined,
  excluding those that died or were sacrificed before week 54.
[a] First adenoma observed at week 104, simultaneously in all dose
  groups at final sacrifice.
[b] First carcinoma observed at week 87, dose 450 ppm.
[C] One animal in the 450 ppm dose group had both an adenoma and a
  carcinoma.
[n] Negative change from control.

Note: There were no liver adenomas or carcinomas in any interim sacrifice animals.
       Significance of trend denoted at <u>control</u>.
       Significance of pair-wise comparison with control denoted at <u>dose</u> level.
       If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Diclofop-Methyl - Wistar (Hoe:WISKf(SPF71)) Study

<u>Female</u> Uterine and Thyroid Gland Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values) (Brunsman, 1999)

	Dose (ppm)			
	0	4.5	45	450
Uterine Glandular Polyps (%)	0/70 (0)	2/69 (3)	1/69 (1)	4[a]/70 (6)
p=	0.033*	0.245	0.496	0.060
Thyroid Gland Follicular Cell Adenomas (%)	1/70 (1)	0/68 (0)	5/67 (7)	5[b]/70 (7)
p=	0.036*	0.507[n]	0.094	0.104

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
[a]First uterine glandular polyp observed at week 97, dose 450 ppm.
[b]First thyroid gland follicular cell adenoma observed at week 104, dose 450 ppm.
[n]Negative change from control.

Note: There were no uterine glandular polyps or thyroid gland follicular cell adenomas in any interim sacrifice animals.
         Significance of trend denoted at <u>control</u>.
         Significance of pair-wise comparison with control denoted at <u>dose</u> level.
         If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5: Historical Control Data for Wistar Rat [Range of % (mean %)] from Pharma Development Corporate Toxicology, Hoechst Aktiengesellschaft, Frankfurt, Germany [a]

Tumor	Male	Female
Liver, Adenoma Carcinoma	0-10% (1.7%) 0-4% (1.7%)	0% (0%) 0-2% (0.8%)
Testes, Leydig cell tumor	5-10% (8.3%)	---
Uterus, Glandular polyps	---	0-2% (0.8%)
Thyroid gland, Follicular cell adenoma	0-2% (1.7%)	0-2% (0.8%)
Adrenal medulla, Adenoma	0-2% (0.8%)	0% (0%)

[a] Studies < 27 months long, 3 studies with 120 animals/sex and 50 or 100 animals/group.

Table 6: Historical Control Data for Wistar Rat [Range of % (mean %)] from Registry of Industrial Toxicology Animal Data, Hannover Germany [a]

Tumor	Male	Female
Liver, Adenoma Carcinoma	0-6% (2.3%) 0-10% (2.1%)	0-5% (0.6%) 0-10% (1.2%)
Testes, Leydig cell tumor	0-52.5% (17.7%)	---
Uterus, Glandular polyps	---	0-8.2% (0.9%)
Thyroid gland, Follicular cell adenoma	0-10.1% (2.4%)	0-4.1% (1.2%)
Adrenal medulla, Adenoma	0-23% (8.3%)	0-34.7% (3.8%)

[a] Studies < 27 months long, 24 studies with 1400 animals/sex and 50 or 20 animals/group.

C. Non-neoplastic Lesions and Other Findings:

At 45 ppm, males and females in the 24-month studies showed signs of liver toxicity, including increased liver weights, impaired lipid and protein metabolism, and increased liver enzyme activity. Related microscopic findings were hepatocellular hypertrophy, epithelial lipofuscin storage, and necrosis; the effects were more pronounced in males. Liver cell enlargement was observed in both sexes at 45 ppm in the 12-month study. Spleen weight was decreased at 450 ppm. Increases in kidney weight in males were significant and dose-related at 45 ppm. Also at 45 ppm, kidneys of both sexes exhibited a shift in lipofuscin storage from focal to diffuse. At the high dose of 450 ppm, body weight gain was significantly reduced in both sexes in the 12-month and two 24-month studies. In the 24-month studies, treatment-related increases in absolute and/or relative liver and kidney weights were observed at the high dose in males and females. In addition to the microscopic liver abnormalities seen from the 45 ppm dose, the high dose produced a significant showing of atypical eosinophilic foci and basophilic foci in both sexes.

Survival analyses indicated no significant incremental changes in mortality with increasing doses of diclofop-methyl in male or female rats.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

A sufficient number of animals were alive at the interim and terminal sacrifices to assess the carcinogenic response of diclofop-methyl. For high-dose (900 ppm) females, dosing was terminated after 24 weeks because of excessive mortality. Body weight gains at 450 ppm was significantly less in males and females, 19% and 27%, respectively after 12 months of treatment and 26% and 37%, respectively, after 24 months of treatment. The microscopic findings observed consisted of hepatocellular hypertrophy, epithelial lipofuscin storage, and necrosis at $\geq$ 45 ppm as well as atypical eosinophilic foci and basophilic foci at 450 ppm in both sexes. Based on these findings, the CARC considered the dosing at the highest dose to be adequate and not excessive. The study was considered to be acceptable/guideline.

III. TOXICOLOGY

A. Metabolism

In the metabolism study (41573306), [dichlorophenoxy ^{14}C]-labeled Hoelon (>98%, 20.82 mCi/g) was administered to male and female Wistar rats (5/sex/group). Test compound was orally administered at a low (0.5 mg ^{14}C-Hoelon/kg), high (5 mg ^{14}C-Hoelon/kg), or repeated low dose (0.5 mg/kg/day x 14 days, followed by 0.5 mg/kg of ^{14}C-Hoelon).

The percent of the radioactivity eliminated over a 96-hour period were quantitatively similar for the low-, high-, and repeat low-dose groups; no differences were noted between sexes. Most of the radioactivity was eliminated in the feces (69.7 to 77.9%) while less was eliminated in the urine (16.4 to 18.9%); no measurable amount (< 0.01%) of radioactivity was found in expired air. Total recovery of ^{14}C-label was between 100.9 and 103.0%.

Tissue levels of radioactivity of low-dose groups were about 7 to 20 times lower than that of the high-dose groups. Within each of the three treatment groups, residual tissue radioactivity, except the ovaries (attributed to the higher fat content), was generally similar for males and females. Residual radioactivity was found mainly in the liver, kidney and digestive tract; the highest levels of radioactivity were found in the fat.

Plasma half-life (t½) of residual radioactivity was similar for low- and high-dose groups, with no differences due to sex (15 hr, males; 14 hr, females). Pharmacokinetic evaluation of the area under the curve (AUC) of high-dose groups (581 to 606 μg x hr/g of plasma) was approximately 10 times higher than that of the low-dose groups (47.0 to 57.5 μg x hr/g of plasma). Peak plasma levels were reached after 4 hr in both the low- (2.05 μg/g of plasma, males; 2.32 μg/g of plasma, females) and high- (21.0 μg/g of plasma, males; 22.1μg/g of plasma, females) dose groups.

The metabolic profiles showed up to eight urinary and six fecal metabolites; no parent compound was identified in either the urine or feces. Two metabolites were identified in both the urine (U) and feces (F): U7, F4 = hydroxylated parent compound [2-(4-(2',4'-dichloro-5-hydroxyphenoxy)phenoxy)propionic acid] and U2, F3 = sulfate conjugate of U7, F4; a third urinary metabolite, U8, was identified as deesterified parent compound [2-(4-(2',4'-dichlorophenoxy)phenoxy) propionic acid]. As percentages of the administered doses, U2 = 10.2 to 13.9% in males and 3.2 to 5.4% in females, U7 = 2.3 to 2.8% in males and 1.6 to 1.9% in females, and U8 = 0.4 to 2.5% in males and 2.9 to 8.3% in females. For the fecal metabolites, F3 = 17.3 to 30.5% in males and 32.2 to 45.7% in females and F4 = 9.4 to 15.4% in males and 3.7 to 6.2% in females. An unidentified fecal metabolite accounted for 8.2 to 9.1% of the administered dose in males and 4.9 to 6.6%, males and 2.1 to 6.4%, in females. The study is Acceptable (Guideline) and fulfills the requirement for a metabolism study (85-1, 870.7485) the rat.

B. **Mutagenicity**

The data indicate that diclofop-methyl is not mutagenic under the testing conditions and there is no mutagenic concern at the present time. Although some of the studies were unacceptable and did not fulfill the guideline requirement, the CARC concluded that no additional testing is required at this time.

1. **Gene Mutations**

Bacterial reverse mutation test in *Salmonella typhimurim*
    870.5100
    MRID 00071904 (HED 000076)
    Dose range: 0 to 5000 μg/mL +/- S9
    Negative for mutagenic effects
    Acceptable (Guideline)

In vitro mammalian cell gene mutation test with Chinese hamster V79 cells

870.5300
MRID 41573305 (HED 008541)
Dose range: 2 to 500 μg/mL +/- S9
Test was negative up to cytotoxic concentrations ($\geq$ 200 μg/mL, -S9; $\geq$ 300 μg/mL, +S9).
Acceptable (Guideline)

2. Cytogenetics

In vitro mammalian chromosomal aberration test in primary human lymphocytes
870.5375
MRID 41476004 (HED 013723)
Dose range: 1 to 500 μg/mL +/- S9
Test was negative up to a cytotoxic concentration (500 μg/mL).
Acceptable (Guideline)

In vivo cytogenetic test in bone marrow cells of the Chinese hamster
870.5385
MRID 41737901 (HED 008850)
Dose range: 0, 200, 1000, and 2000 mg/kg
Chromosomal analysis did not show any treatment-related cytogenetic aberrations up to the highest dose tested.
Acceptable (Guideline)

3. Other Genotoxic Effects

Unscheduled DNA synthesis in primary rat hepatocytes *in vitro*
870.5550
MRID 00087816 (HED 001422)
Dose range: 0.5 to 50 μg/mL, cytotoxicity at 100 μg/mL
Did not induce significant increases in nuclear labeling of primary rat hepatocytes up to a cytotoxic concentration.
Acceptable (Guideline)

Unscheduled DNA synthesis in A549 human lung carcinoma *in vitro*
870.5550
MRID 41996902, 42437801 (HED 008796)
Dose range: 0.03 to 100 μg/mL $\pm$ S9
Did not induce significant increases in nuclear labeling human lung cancer cells up to the highest dose tested.
Acceptable (Guideline)

4. Other submitted data, but classified as unacceptable

Dominant lethal test in male NMRI mice
    870.5450
    MRID 00071906 (HED 000076)
    Dose range: 0, 10, 32, 100 mg/kg/day
    Did not induce dominant lethal effects up to the
    highest dose tested. However, the test was not conducted at the maximum tolerated dose.
    Unacceptable (Guideline)

Mouse bone marrow micronucleus test
    870.5395
    MRID 00071905 (HED 000076)
    Dose range: 0, 10, 32 and 100 mg/kg
    Negative, no increase in incidence of polychromatic erythrocytes with micronuclei up to the
    highest dose tested. However, the test was not conducted at high enough dosing, no positive
    controls were used and the study protocol was inappropriate.
    Unacceptable (Guideline)

Mutagenic activity with *Saccharomyces cerevisiae*
    870.5575
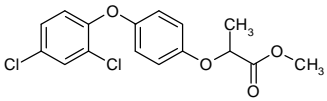   MRID: 00087820
    Dose range: 0.25 - 1000 μg/mL
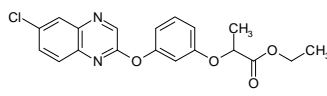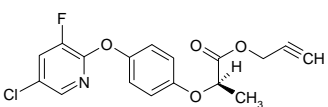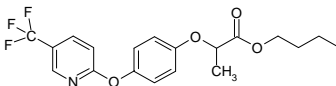   Negative for gene conversion. However, the concentrations used were not high enough.
    Unacceptable (Guideline)

C. **Structure-Activity Relationship**:

There are eight diphenyl ethers that are structurally similar to diclofop-methyl. Of the chemicals,
fomesafen sodium, haloxyfop-methyl (Verdict), oxyfluorfen, acifluorfen sodium, nitrofen, and
lactofen were reviewed in the initial CPRC report. All of these chemicals induced liver adenomas
and carcinomas in rats and/or mice. Except for haloxyfop-methyl, all of the other chemicals
produced positive results in at least one of the mutagenicity assays.

Since the initial committee meeting, three additional diphenyl ethers, clodinafop-propargyl,
fluazifop-butyl, and quizalofop-ethyl, were identified and the data are presented below.

Table 9: Carcinogenic Effects of Diclofop-methyl and Related Diphenyl Ethers

Compound	Structure	Carcinogenic Effect
Diclofop-Methyl 　PC Code: 110902 　CAS: 51338-27-3		Classification: Likely Hepatocellular adenoma and carcinomas in NMRKf (SPF) mice $Q1^* = 2.3 \times 10^{-1}$ Not mutagenic
Quizalofop-Ethyl 　PC Code: 128711 　CAS: 76578-14-8		Classification: D Negative in UDS and Ames assays
Clodinafop-Propargyl 　PC Code: 125203 　CAS 105511-96-4		Classification: Likely Prostate and ovarian adenomas and carcinomas in rats and liver adenomas and carcinomas in Tif: RAIf (SPF) mice Not mutagenic
Fluazifop-butyl 　PC Code: 122805 　CAS: 69806-50-4		No classification No evidence of carcinogenicity in rat (acceptable) or mouse (unacceptable, no MTD) studies. Negative Ames assay

D. Subchronic Toxicity

Subchronic Oral Toxicity Feeding - Rat: In this subchronic toxicity study(42573301), Wistar rats (20/sex/dose) were fed diets containing diclofop methyl at concentrations of 0 (basal diet), 5, 20, 80, or 320 ppm (0, 0.34, 1.6, 6.3, or 26 mg/kg/day, males; 0, 0.44, 1.8, 7.1, 28 mg/kg/day, females) for 13 weeks, followed by a four-week, treatment-free (basal diet) recovery period with 10 rats/sex in the control, 80, and 320 ppm groups.

No treatment-related clinical signs or deaths occurred during the study. Males dosed at 320 ppm, had significantly ($p \leq 0.05$) decreased mean body weights (9-11%) and body weight gains (12 to 14%) at 46 and 92 days of treatment; body weights were decreased (8.4%, not significant) at 120 days. At the end of the recovery period, the body weights of high-dose males were still reduced (9%, not significant). The body weights of females were unaffected by treatment. Food consumption by high-dose males were decreased 4 to 14% (not significant) during the study. For the remaining treatment groups, food and water consumption were comparable to the control groups of both sexes.

Clinical pathology revealed treatment-related changes in some hematological and clinical chemistry parameters. Coagulation times and thromboplastin times were significantly decreased (19% and 31%, respectively) in high-dose males, but returned to control levels at the end of the recovery period. Clinical chemistry effects in 80 and 320 ppm males included decreases in cholesterol (29 and 45%, respectively) and total lipids (26 and 42%, respectively); free fatty acids were decreased in 80 ppm females (28%) and high-dose males (55%) and females (19%). In males, AST (SGOT) and ALT (SGPT) were increased at 320 ppm (20 and 30%, respectively); alkaline phosphatase was increased at 80 and 320 ppm in males (38 and 61%, respectively) and females (42 and 46%, respectively). At the end of the recovery period, only free fatty acid levels were still decreased at 80 and 320 ppm in males (26 and 23%, respectively) and females (24 and 20%, respectively).

Selected liver enzymes, used as indirect biomarkers for peroxisome proliferation and microsomal enzyme induction were measured at the end of the main study and recovery period. Assay of liver homogenates showed increases in malic enzyme and catalase, both indirect enzyme markers for peroxisomal proliferation. Malic enzyme was significantly increased in 80 ppm and 320 ppm males (65 and 96%, respectively) and 320 ppm females (42%). Catalase was significantly increased in 5, 20, and 80 ppm females (85 to 189%) and in 320 ppm males (146%) and females (474%). Microsomal enzymes (glycerophosphate dehydrogenase, $NADPH_2$-dependent cytochrome c reductase and glucuronyltransferase) were induced in 80 ppm females and/or 320 ppm males and females; ethylresorufin o-deethylase activity was decreased in 80 ppm males and 320 ppm males and females. At the end of the recovery period, there was no increase in enzyme activity associated with peroxisome proliferation; glucuronyltransferase activity was still increased in 80 and 320 ppm males and females.

Treatment-related pathological changes were limited to the liver. Absolute and relative liver weights were significantly increased in 80 and 320 ppm males and absolute liver weights, in 320 ppm females. Histopathological evaluations revealed centrilobular with marked cytoplasmic granulation (suggestive of peroxisome proliferation). Electron micrographs of the high-dose animals (1/sex) showed an increase in peroxisomes associated with an increase in smooth endoplasmic reticulum The study is classified as acceptable (guideline).

Subchronic Oral Toxicity Feeding - Mouse: In a subchronic toxicity study (MRID 42593901) diclofop-methyl was administered to 10 - 15 NMRI mice/sex/dose at the dose levels of 0, 2, 6.3, 20, and 63 ppm (0, 0.3, 1.0, 3.3, 10.4 mg/kg/day, males; 0, 0.4, 1.2, 3.8, 12.4 mg/kg/day, females) for 13 weeks. At the end of treatment, 5 mice/sex, in the control, 20, and 63 ppm doses groups, were carried over to a 4-week, treatment-free (basal diet) recovery phase.

There were no treatment-related clinical signs or deaths during the study. Males dosed at 63 ppm, had significantly decreased mean body weights (8-11%) and body weight gains (16 to 18%) after 44 and 92 weeks of treatment; at the end of the recovery period, the body weights of control and high-dose males were comparable. The body weights of females were unaffected by treatment. Feed consumption was comparable between treated and control groups.

Treatment-related changes in some hematological and clinical chemistry parameters, as well as changes in activities of selected enzymes in liver homogenates, were observed. Coagulation times in high-dose males were significantly decreased (29%) after 13-weeks of treatment, but returned to control values after the recovery period. In males, triglyceride and total lipids were increased at 6.3 ppm and higher doses; AST, ALT, and ALP were increased at 20 ppm and higher. For females, cholesterol and ALP activities were increased at 20 ppm and higher, while increases in triglycerides and ALT (SGPT) were observed only at the high-dose. At the end of the recovery period, clinical chemistry parameters, except for ALT activities in females, returned to control values.

Assay of liver homogenates showed increases in malic enzyme and catalase, both indirect enzyme biomarkers for peroxisomal proliferation. Malic enzyme was increased in all treated male groups (101 to 785%) and females dosed at 6.3 ppm and higher(50 to 350%); catalase was increased in 6.3 ppm males (484%) and 20 and 63 ppm males (785 and 367%, respectively) and females (446 and 357%, respectively). At the end of the recovery period, catalase (males and females) and malic enzyme (females only) returned to control values. Microsomal enzymes (NADPH$_2$-dependent cytochrome c reductase and/or glycerophosphate dehydrogenase) were induced in all treated males and/or females. At the end of the recovery period, increased activity was still observed at the high-dose level in both sexes.

Treatment-related pathological changes were limited to the liver. Absolute and relative liver weights were significantly increased in a dose-dependent manner at 6.3 ppm and higher. Dose-related increases in single cell necrosis were observed in both sexes at 2 ppm and higher. Kupffer cell proliferation, enlargement of centrilobular hepatocytes with loss of basophillia (an indication of microsomal enzyme induction), and liver cell mitosis were observed in males dosed at 6.3 ppm and higher and females dosed at 20 ppm and higher. These three hepatic lesions were observed in essentially all of the high-dose animals. Increased liver cell mitosis was observed in males dosed at 6.3 ppm and females dosed at 63 ppm.

The LOAEL was established at 2 ppm (0.3 mg/kg/day, males; 0.4 mg/kg/day, females) based upon hepatotoxicity including increases in peroxisomal proliferation, microsomal enzyme induction, and liver necrosis. The NOAEL was not established. The study is classified as acceptable (guideline).

Repeated Dose (21-Day) Dermal – Rat : In a repeated-dose dermal toxicity study (MRID 41476004), diclofop-methyl (Hoe 023408 OH ZD95 0003 Technical; 94.5% a.i.) was applied to the clipped intact skin of 11 Wistar rats/sex/dose at nominal dose levels of 0 (diluent-treated control), 5, or 125 mg/kg/day (<limit dose), and to the clipped intact skin of six Wistar rats/sex/dose at a nominal dose level of 25 mg/kg/day for 6 hours/day, 5 days/week, for a total of 21 applications during a 30-day period. Five rats/sex in the control, 25, and 125 mg/kg/day groups were maintained for a 4-week recovery period to determine the reversibility of effects.

All animals survived the 30-day study. No treatment-related signs of dermal toxicity were observed. No treatment-related differences in toxicity, body weights, food consumption, urinalysis, or gross pathology were observed between the control and treated groups, and no

neoplastic tissue was observed. Signs of systemic toxicity were observed in the livers of the 25 and 125 mg/kg/day animals.

At 25 mg/kg/day, absolute and relative liver weights were increased (males, each 35%; females, 20-21%; $p \le 0.05$) when compared to concurrent controls. Males displayed dose-dependent increases ($p \le 0.05$) in mean ALP activity (32%) and gamma$_1$-globulins (8%) and females displayed a dose-dependent increase in alpha$_1$-globulin levels (21%; $p \le 0.05$).

At 125 mg/kg/day, the liver and lipid metabolism were adversely affected. Enlarged (not tested for statistical significance) centrilobular hepatocytes were observed in 5/11 males and 3/11 females vs 0/22 controls (mean enlargement, 20.3% in males; 45.4% in females) and remained enlarged (20%) in females at the end of the recovery period. Absolute and relative liver weights were increased ($p \le 0.05$) in males (53% and 60%, respectively) and females (57% and 48%, respectively) at the end of the treatment period and remained higher in the females (14-17%; $p \le 0.05$) following the recovery period. Males displayed ($p \le 0.05$) increased mean ALP activity (42%) and decreased cholesterol levels (30%) and females exhibited ($p \le 0.05$) a prolonged activated partial thromboplastin time (299%) and increased levels of triglycerides (43%), total protein (12%), and alpha$_1$-globulin (22%).

The systemic LOAEL is 25 mg/kg/day, based on increased liver enzymes, proteins, and absolute and relative liver weights. The systemic NOAEL is 5 mg/kg/day. The dermal LOAEL was not observed. The dermal NOAEL was ≥ 125 mg/kg/day. This study is classified as acceptable (guideline).

E. **Chronic Toxicity**

Chronic Toxicity/Carcinogenicity Study - Rat (MRID 43927302): Refer to discussion on p. 2 and 5 for details. The LOAEL for systemic toxicity is 45 ppm in male (2.32 mg/kg/day) and female (3.05 mg/kg/day) rats, based on liver toxicity manifested as increased organ weight, impaired lipid and protein metabolism, increased enzyme activity, hepatocellular hypertrophy, and increased epithelial lipofuscin storage, and increased kidney weight and a shift from focal to diffuse lipofuscin storage pattern. The NOAEL is 4.5 ppm in males (0.23-0.27 mg/kg/day) and females (0.3 mg/kg/day).

Chronic Feeding Study - Rat: In this combined chronic toxicity/oncogenicity study (MRID 92036057), rats were fed diets containing diclofop-methyl at concentrations of 0, 2.0, 6.3, or 20 ppm (0, 0.1, 0.32, 0.99 mg/kg/day, males; 0, 0.12, 0.39, 1.25 mg/kg/day, females) for 24 months.

No treatment-related clinical effects were observed during the study. After 24 months of treatment, mortality of low- and mid-dose males and all treated female groups were comparable to control values; mortality of high-dose males was less than the control value. Body weights of all male treatment groups and low- and mid-dose females were comparable to control values; for high-dose females body weights were slightly decreased (4 to 9%) from week 26 through 78. The decrease observed in high-dose females was not considered

toxicologically significant and not sufficient to establish this finding as an MTD.

Clinical pathology results showed effects in hematology and clinical chemistry; urinalysis values were not affected by treatment. Decreases in hemoglobin concentration in mid- and high-dose males at weeks 28 and 53, respectively; hemoglobin concentrations at 79 and 104 weeks were comparable to control values. Females showed sporadic changes in hemoglobin concentrations through week 79; at week 104, decreases were noted in all of the treated female groups. Consistent clinical chemistry effects were observed at 20 ppm, and included decreased cholesterol and total lipids and increased ALT (SGPT).

At terminal sacrifice, increased organ weights and increased incidence of non-neoplastic findings were observed. For females, increases in relative heart, liver, and kidney weights were observed at 6.3 ppm, and relative thyroid, adrenals and liver weights at 20 ppm. Organ weights of low-dose females and all treated male groups were unaffected by treatment. Non-neoplastic lesions were observed in mid- and high-dose males, where increased incidence of foam cell aggregation (34% and 49%, respective to dose) and cholesterol clefts (7.1% and 13%, respective to dose) were observed in the lungs. These lesions in the lung may be an age-related finding and not a treatment-related effect of diclofop-methyl. High-dose males showed an increased incidence (7.3%) of craniopharyngeal cysts in the pituitary, while high-dose females showed increased incidence of uterine lesions (cystic endometrial hyperplasia, 11.6% and endometrial polyps, 16.2%).

The LOAEL for chronic toxicity was established at 6.3 ppm (0.32 mg/kg/day), based on increased relative organ weights (heart, liver, kidneys) in females and increased incidence of histopathological lesions (foam cell aggregation and cholesterol clefts in lungs) in males. The NOAEL was established at 2 ppm (0.1 mg/kg/day). The study is classified as acceptable (guideline).

F. Mode of Action Studies

An International Life Sciences Institute (ILSI) workshop listed several criteria which were considered necessary for a compound to be classified as a peroxisome proliferating hepatocarcinogen (Cattley, R., 1998). These criteria include: 1) Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and increased number of peroxisomes as measured by morphometric analysis; 2) Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy; 3) Increased levels of enzymes involved in peroxisomal fatty acid metabolism, especially acyl or palmitoyl CoA oxidase activities.

Review of the toxicology database for diclofop-methyl indicates that only two of the three criteria have been met; there were no data to demonstrate increased DNA synthesis using BrdU labeling. In addition to an evaluation of these criteria as they apply to diclofop-methyl, livers

of treated animals were examined under an electron microscope.

In all subchronic, chronic, carcinogenicity, developmental toxicity and reproductive toxicity studies in the rat and/or mouse the liver was the target organ of toxicity.   In these, increased liver weights and centrilobular hypertrophy were consistently observed.

In the subchronic feeding studies in the rat and mouse, the standard protocol was modified to include measurement of peroxisome-specific enzymes - catalase and malic enzyme- in liver homogenates of diclofop-methyl treated animals. In both studies, main study animals were treated for 13 weeks, with a subset of animals carried through into four-week, treatment-free recovery period. In both rats and mice, the activities of malic enzyme and catalase were markedly increased (330 - 450% and 540 - 880%, respectively) at the end of the main study. At the end of the recovery period, the activity of these enzymes returned or was returning to control values.

In the subchronic feeding study in the rat and carcinogenicity study in the mouse livers were examined both histologically and under electron microscopy. Histopathological examination revealed that essentially all mid- and high-dose male rats and high-dose female rats showed enlargement of centrilobular liver cells, and according to the pathology report, the enlarged liver cells showed conspicuous granulation of the cytoplasm, suggestive of peroxisome proliferation. The livers of one high-dose rat/sex were further evaluated under an electron microscope, which showed an increase in peroxisomes associated with an increase in smooth endoplasmic reticulum. In the mouse carcinogenicity study, centrilobular hypertrophy was also evident. Electron microscopy of liver cells (details not given in study) showed a marked proliferation of peroxisomes of considerably varying size in the high-dose group.

Recent publications show strong evidence and scientific consensus that peroxisome proliferation is directly associated with the onset of liver cancer in rodents (Doull, J. et al., 1999 and Lupinskas, P.J. and Corton, J.C., 1997).   Of the mechanisms proposed for the formation of hepatocellular tumors in rodents, peroxisome proliferation is the most widely accepted and scientifically valid. The triggering mechanism for peroxisome proliferation is the binding of a peroxisome proliferator, either naturally occurring (fatty acids, prostaglandins, endogenous steroids) or man-made (industrial plasticisers, hypolipidemic fibrate drugs, chlorinated hydrocarbons, or diphenyl ether pesticides) to the peroxisome proliferator-activated receptor (PPAR). PPARs, of which four subtypes have been identified (α, β, γ, and δ), are transcription factors of the steroid hormone receptor superfamily. Of the four PPARs, α, γ and δ subtypes are present in both rodents and humans. The effects of peroxisome proliferators are mediated by PPARα in rodents; PPARα is refractory in human liver.   To become fully active, the peroxisome proliferator-PPAR complex forms a heterodimer with 9-retinoic acid-retinoid X receptor complex. The activated PPARα interacts with specific response elements (PPREs), located upstream of a series of genes. These genes

include, in part, those responsible for the fatty acid β-oxidation enzyme system (particularly, acyl CoA oxidase) and other unidentified genes responsible for growth and hepatocarcinogenesis.

There is strong evidence that peroxisome proliferation and non-mutagenic hepatocarcinogenicity is limited to rodents. Many *in vivo* experiments have been conducted which show that peroxisome proliferators are responsive in the rat and mouse and non-responsive in the dog, rhesus monkey, and marmoset; guinea pigs showed intermediate responsiveness. Studies in humans treated with hypolipidemic drugs (gemfibrozil, clofibrate, fenofibrate), all peroxisome proliferators in rodents, did not show peroxisome proliferation in liver biopsies (Hertz, R. and Bar-Tana, J, 1998). *In vitro* experiments also showed clear species differences in cultured rodent and human hepatocytes. Incubation of isolated hepatocytes with phthalic acid esters demonstrated a clear response (increased DNA synthesis and decreased apoptosis) in rats, but no similar responses in humans (Hasmall, S.C. et al., 1999) .

Perhaps the most compelling evidence to support the cause-and-effect relationship between peroxisome proliferation and non-genotoxic hepatocarcinogenicity comes from recent experiments with transgenic mice (Lee *et al.* , 1995). In one series of experiments, transgenic mice, which lacked expression of PPARα mRNA, were treated with a known peroxisome proliferator, Wy-14,643. While feeding of Wy-14,643 to wild-type mice resulted in the development of multiple tumors, the transgenic mice were unresponsive. Another study used transgenic mice devoid of peroxisomal fatty acyl CoA oxidase. In these mice, the PPARα receptor is continuously occupied by fatty acyl CoA leading to the development of severe peroxisomal proliferation and increased mRNA levels of PPARα-regulated genes. Further, these mice developed hepatocellular adenomas and carcinomas.

In summary, peroxisome proliferation is a receptor-based activation of PPAR by a peroxisome proliferator. This mechanism is active in rodents, while humans are seemingly non-responsive (Hertz, R. and Bar-Tana, J., 1998; Hasmall, S. C. et al., 1999). The lack of human response may be explained by either the low concentrations of PPARα or lower expression levels PPARα.

MTARC's conclusion Regarding the Mode of Action

On March 29, 2000, a joint meeting of the Health Effects Division's Mechanism of Toxicity Assessment Review Committee (MTARC) and CARC met to discuss mode of action on liver carcinogenicity of Diclofop-methyl. The Registrant contended that diclofop-methyl is a rodent-specific peroxisome proliferator which manifests itself in the induction of peroxisomal and microsomal enzymes resulting in tumor formation.

! At the joint meeting, the data from the submitted studies discussed above as well as the data from two recent open literature publications were reviewed. The data on structurally-related diphenyl ethers were also reviewed. For fomesafen (P.C.Code 123802) the data included, a 4-week feeding study in the mouse (MRID 40786709), a 28-day feeding study in the hamster (MRID 40910801) and an in vitro study (MRID 40910802, Elcombe et al., Annals NY Acad Sci. 804: 628-635 [1996]) with rat, mouse, guinea pig and human hepatocytes. For haloxyfop (P.C. Code 125202), a 2/4-week feeding study in the mouse and rat [Stott et al., Fund. Appl. Toxicol. 28: 71-79 (1995)] was reviewed. The

mechanism of action of diclofop-methyl was evaluated using the criteria discussed in the ISLI workshop (Cattley, R., 1998) to support characterization of a nonmutagenic hepatocarcinogenic substance as a peroxisome proliferator. Review of the toxicology database for diclofop-methyl indicates that the criteria were generally addressed. However, there were following shortcomings in the data base:

(1) There were not actual measurements of the number of peroxisomes using morphometric analysis and there was a lack of dose-response information.

(2) The studies measured catalase activity instead of measurement of acyl or palmitoyl CoA oxidase activities which would have served as sensitive indicators of peroxisome proliferation.

(3) Quantification of hepatic peroxisome proliferation in rats/mice and proliferation of smooth endoplasmic reticulum were not evaluated. The studies were limited to electron micrographic evidence for peroxisome proliferation of high-dose animals (one animal/sex) from a mouse carcinogenicity study and a 90-day feeding study in the rat. Control or intermediate dose animals were not examined, which makes evaluation of possible dose-response relationships impossible to determine.

(4) The dose-response for hepatic DNA synthesis after short-term and longer-term treatment with diclofop-methyl and reversibility of effects after ending treatment were not addressed.

The MTARC and CARC agreed that there is some evidence of peroxisome proliferation in the submitted studies because effects indicative of peroxisome proliferation were observed in both rats and mice. Effects were consistent between species and included dose-dependent increases in relative liver weights and hepatic catalase activities. The data for diclofop-methyl were consistent with effects observed with two structurally related chemicals, haloxyfop and fomesafen.   However, the studies lack the depth and quality to establish peroxisome proliferation as the mode of action for non-mutagenic hepatocarcinogenicity of diclofop-methyl (for details refer to attachment for MTARC, 2000).

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

1. Carcinogenicity:

!   **The CARC concluded that diclofop-methyl was carcinogenic in male and female Wistar rats**.

In male rats, there were significant ($p<0.01$) differences in the pair-wise comparisons of the 450 ppm group with the controls for hepatocellular adenomas, carcinomas and

combined adenomas/carcinomas (14/69, 20% vs 0% in controls; carcinomas; 18/69, 26% vs 0% in controls ; combined: 29/69, 42% vs 0/70, 0% in controls).   Significant (p<0.01) increasing trends were also evident for these tumors.   The incidence of adenomas and carcinomas exceeded the historical control range (0%-10% and 0%-4%, respectively). The CARC considered these tumors to be treatment-related. In addition, a significant increasing trend was noted for testicular Leydig cell tumors (p<0.05) and the occurrence of these tumors was preceded by hyperplasia of Leydig cells. The Committee considered this finding to be possibly treatment-related.

! **In female rats**, there was a significant increase by pair-wise comparison of the 450 ppm dose group with the controls for hepatocellular carcinomas and combined adenomas/carcinomas (carcinomas: 14/70, 20% vs 1/70, 1% in controls; combined: 19/70, 27% vs 2/70, 3% in controls).   The increase in adenomas was of borderline significance (p=0.05; 6/70, 9% vs 1/70, 1% in controls). Significant (p<0.01) increasing trends were evident for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. The incidence of adenomas and carcinomas exceeded the historical control range (0% and 0%-2%, respectively). The CARC considered these tumors to be treatment-related. In addition, a significant (p<0.05) increasing trend was noted for uterine glandular polyps and thyroid follicular cell adenomas. The Committee considered these tumors to be supportive of carcinogenic potential of diclofop-methyl.

The dosing at 450 ppm was considered to be adequate and not excessive based on decrease in body weight gain, and histopathological changes in the liver in both male and female rats.

! **Diclofop-methyl induced hepatocellular adenomas, carcinomas and combined adenomas/carcinomas in male and female B6C3F1 mice (CPRC, 1993).** In the light of the Agency's *Draft Cancer Risk Assessment guidelines* (July, 1999), the mouse study was reevaluated by the CARC.   The majority of the CARC members (10 vs 1) determined that the dosing at the highest dose was adequate and not excessive in both sexes based on changes in clinical chemistry, organ weight and histopathological changes in the liver. Although there was a significant increase in mortality in high dose males, the study results did not compromise the qualitative assessment. One of the Committee members considered the high dose in males to be excessive based on the presence of liver necrosis and high mortality. The CARC reaffirmed the CPRC's decision that diclofop-methyl was a hepatocarcinogen in both sexes of mice.

The CARC concluded that the new acceptable rat study supports the CPRC's assessment of the carcinogenic potential of diclofop-methyl in that it displays evidence for carcinogenicity in rats as well as in mice.

2. **Mutagenicity**

!   The review of both *in vitro* and *in vivo* mutagenicity studies indicate a non-mutagenic effect.

3. Structure Activity Relationship

!   The diphenyl ethers which are structurally similar to diclofop-methyl (including fomesafen sodium, haloxyfop-methyl (Verdict), oxyfluorfen, acifluorfen sodium, nitrofen, lactofen, clodinafop-propargyl, fluazifop-butyl, and quizalofop-ethyl) are either liver toxins or induce liver tumors in rats and/or mice. Except for haloxyfop-methyl, all of the other chemicals produced positive results in at least one of the mutagenicity assays.

4. Mode of Action

!   **In a joint meeting, the MTARC and CARC agreed that some evidence of diclofop-induced peroxisome proliferation was observed in both rats and mice. The data for diclofop-methyl were also consistent with effects observed with two structurally related chemicals, haloxyfop and fomesafen.   However, the available studies on diclofop-methyl were inadequate to establish peroxisome proliferation as the mode of action for its hepatocarcinogenicity.**

V. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (EPA, 1999), the CARC classified diclofop-methyl as **"likely to be carcinogenic to humans"** by the oral route based on the following weight-of-the-evidence considerations:

1. Liver tumors were seen in both sexes of two species including both benign and malignant liver tumors in rats and mice. Increases in the incidence of thyroid follicular cell tumors in female rats and Leydig cell tumors in male rats were possibly treatment-related.

2. The relevance of the observed tumors to human exposure cannot be discounted.

3. Diclofop-methyl is not mutagenic in both *in vivo* and *in vitro* assays.

4. Structurally related diphenyl ethers cause liver tumors in rats and/or mice. Some of these compounds such as clodinafop-propargyl and lactofen, are also peroxisome proliferators.

VI. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose (Q_1*) extrapolation approach for the quantification of human cancer risk based on the most potent of the liver tumors observed in mice. This approach is supported by the lack of confirmation of the mode of action.

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April 19, 2000

MEMORANDUM

SUBJECT: **DICLOFOP-METHYL**: Report of the Mechanism of Toxicity Assessment
     Review Committee

FROM: Robert F. Fricke
     Reregistration Branch II
     Health Effects Division (7509C)

THROUGH: Karl Baetcke,
     MTARC, Chairman,
     Health Effects Division (7509C)

TO: Christina Jarvis
     Risk Assessor
     Reregistration Branch II
     Health Effects Division (7509C)

CHEMICAL: Diclofop-methyl, Pesticide Chemical Code: 110902

On March 29, 2000, a joint meeting of the Health Effects Division's Mechanism of Toxicity
Assessment Review Committee (MTARC) and Cancer Peer Review Committee met to discuss
mode of action on liver carcinogenicity of Diclofop-methyl. Members present were:
Karl Baetcke, William Burnam, Ghazi Dannan, Keary Dearfield, Stephen Devito, Vicki Dellarco,
Sanju Diwan, Virginia Dobozy, Karen Hammernick, Richard Hill, Yiannakis Ioannou, Penny-
Fenner Crisp, Nancy McCarroll, Esther Rinde, Joycelyn Stewart, Clark Swentzel, Linda Taylor,
John Pletcher, Yung Yang, Pauline Wagner and Yin-Tak Woo.

 Data were presented by Robert Fricke of Reregistration Branch 2.

The committee reviewed data from the following studies (MRID No.) submitted by the Registrant:
30-day range-finding studies in the mouse (088559) and rat (00134486), 90-day feeding studies in
the mouse (42593901) and rat (42593902, 42573301), an oncogenicity study in the mouse
(92036058), and a combined chronic feeding oncogenicity study in the rat (43927302). A
summary (43927201) of pertinent data on the oncogenic weight-of-evidence was also submitted.

Data for structurally-related diphenyl ethers were also reviewed. For fomesafen (P.C.Code
123802), a 4-week feeding study in the mouse (40786709), a 28-day feeding study in the hamster
(40910801) and an in vitro study (40910802, Elcombe et al., Annals NY Acad Sci. 804: 628-635

(1996) with rat, mouse, guinea pig and human hepatocytes. For haloxyfop (P.C. Code 125202), a 2/4-week feeding study in the mouse and rat [Stott et al., Fundam. Appl. Toxicol. 28: 71-79 (1995)].

In addition two recent open literature publications were reviewed. One which summarizes the findings of an ILSI workshop on peroxisome proliferation and human risk assessment [Cattley et al. Regul Toxicol. Pharmacol. 27: 47-60 (1998)] and the other, a summary of the cancer risk assessment of DEHP [Doull et al., Regul Toxicol. Pharmacol. 29: 327-357 (1999)].

Issues discussed are as follows.

A. <u>Proposed Mode of Action</u>

The mechanism of action of diclofop-methyl were evaluated using the criteria discussed in the ISLI workshop to support characterization of a nongenotoxic hepatocarcinogenic substance as peroxisome proliferator. These criteria are:

   1.) Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and increased number of peroxisomes as measured by morphometric analysis.

   2.) Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

   3.) Increased levels of enzymes involved in peroxisomal fatty acid metabolism, especially acyl or palmitoyl CoA oxidase activities.

<u>**Weakness of the submitted data in supporting mode of action on peroxisome proliferation**</u>:

   1.) Although hepatomegaly, as measured by increased relative liver weight, was a consistent finding in both rats and mice, no evidence was presented on actual measurement of the number of peroxisomes using morphometric analysis.

   2.) Increased catalase activities were used as evidence of peroxisome proliferation. The committee felt that measurement of acyl or palmitoyl CoA oxidase activities would be more sensitive indicators of peroxisome proliferation.

   3.) Quantification of hepatic peroxisome proliferation in rats/mice and prolifertion of smooth endoplasmic reticulum needs to be evaluated. For diclofop-methyl, studies were limited to electron micrographic evidence for peroxisome proliferation of high-dose animals (one/sex) from a mouse oncogenicity study and a 90-day feeding study in the rat. Control or intermediate dose animals were not examined, which makes evaluation of possible dose-response relationships impossible to determined.

   4.) The data for diclofop-methyl are not at par with that of fomesafen, which included

measurement of hepatic palmitoyl CoA oxidase activities and determination of peroxisome volume using morphometric analysis.

    5.) Dose-response on hepatic DNA synthesis after short-term and longer-term treatment with diclofop-methyl and reversibility of effects after ending treatment need to be addressed.

Strengths of the submitted data in supporting mode of action on peroxisome proliferation

    1.) Observed effects indicative of peroxisome proliferation were observed in both rats and mice. Effects were consistent between species and included dose-dependent increases in relative liver weights and hepatic catalase activities.

    2.) Data for diclofop-methyl were consistent with effects observed with two structurally related chemicals, haloxyfop and fomesafen.

B.   Other issues discussed:

    None

Conclusion

Although the committee agreed that there is some evidence of peroxisome proliferation, the submitted studies lack the depth and quality to unequivocally establish peroxisome proliferation as the mechanism of action for non-genotoxic hepatocarcinogenicity of diclofop-methyl.   The studies submitted were dated and do not take advantage of more recent methodology (e.g. measurement of more sensitive hepatic enzymes, morphometric analysis of peroxisomes). Further, there was no direct measurement of cell proliferation; the committee felt that time-dose effects on DNA synthesis would establish the sequence of events associated with peroxisome proliferation.