Attachment 3

013893

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

December 7, 1999

MEMORANDUM

SUBJECT: Clodinafop-Propargyl (CGA 184927) - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Stan Gross, Pharmacologist
Registration Action Branch 3
Health Effects Division (7509C)

Anna Lowit, Risk Assessor
Registration Action Branch 3
Health Effects Division (7509C)

The Cancer Assessment Review Committee met on September 29, 1999 to evaluate the carcinogenic potential of Clodinafop-Propargyl. Attached please find the Final Cancer Assessment Document.

cc: K. Baetcke
L. Brunsman
W. Burnam
M. Copley
V. Dobozy
R. Hill
Y. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor

013893

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF **CLODINAFOP-PROPARGYL (CGA 184927)**

FINAL REPORT

7-DECEMBER, 1999

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Yung Yang, Toxicologist

DOCUMENT PREPARATION: Sanjivani Diwan, Executive Secretary

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

Karl Baetcke

William Burnam

Marion Copley

Virginia Dobozy

Richard Hill — See attached sheet

Yiannakis Ioannou

Nancy McCarroll

Linda Taylor

Esther Rinde

Jess Rowland

Joycelyn Stewart

Clark Swentzel

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

Lucas Brennecke, Pathology Consultant — See attached sheet

Lori Brunsman, Statistical Analysis

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Richard Hill	Richard N Hill
Yiannakis Ioannou	______________________
Nancy McCarroll	______________________
Linda Taylor	______________________
Esther Rinde	______________________
Jess Rowland	______________________
Joycelyn Stewart	______________________
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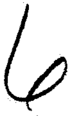

EXECUTIVE SUMMARY

On September 29, 1999, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of clodinafop-propargyl (CGA-184927). The studies evaluated included a 24-month chronic toxicity/carcinogenicity study in Tif: RAIf (SPF) albino rats and an 18-month carcinogenicity study in Tif:MAGf (SPF) albino mice as well as mechanistic studies submitted by the Registrant to support a non-linear mode of action for induction of prostate, ovarian, and liver tumors.

In the above studies, CGA-184927 was administered in the diet to rats (80/sex/group) at 0, 1, 10, 300 or 750 ppm (0, 0.031, 0.32, 10.18, or 26.28 mg/kg/day for males; and 0, 0.034, 0.36, 11.31, or 29.48 mg/kg/day for females, respectively) and to mice (60/sex/group) at 0, 1, 10, 100 or 250 ppm (0, 0.113, 1.10, 11.0 or 29.6 mg/kg/day for males and 0, 0.129, 1.25, 12.6 or 33.1 mg/kg/day for females, respectively).

- **CGA-184927 was carcinogenic to rats** because: 1) In males there were significant increases in the pair-wise comparisons of the high-dose group (750 ppm or 26.28 mg/kg/day) with controls for prostate gland adenomas ($p<0.05$) and combined adenomas/carcinomas ($p<0.01$). There were also significant increasing trends for prostate adenomas and combined adenomas/carcinomas; 2) Females had a statistically significant ($p<0.05$) increased incidence at 750 ppm (29.5 mg/kg/day) by pair-wise comparison with the controls for ovarian tubular adenomas. There was also a statistically significant ($p<0.01$) increasing trend for these tumors. The dosing at the highest dose in males was considered to be adequate based on increased mortality, increased liver weights and non-neoplastic changes in various organs. The CARC considered the prostate and ovarian tumors to be treatment-related.

- **CGA-184927 was carcinogenic to mice** because: 1) Males had a statistically significant ($p<0.01$) increase in the pair-wise comparisons of the high dose group (250 ppm or 29.6 mg/kg/day) with the controls for hepatomas and combined hepatomas/carcinomas. The incidences of these tumors exceeded the range of historical controls. The increased incidence of carcinomas in high-dose males was considered by the CARC to be biologically significant. There were also statistically significant increasing trends for hepatomas ($p<0.01$), carcinomas ($p<0.05$), and combined hepatomas/carcinomas ($p<0.01$); 2) In females, the incidences of liver tumors were not significant by pair-wise comparison with controls and were within the historical control range. However, there were statistically significant increasing trends in hepatomas ($p<0.01$), and combined hepatomas/carcinomas ($p< 0.05$); 3) In females, there was a borderline increase in hemangiomas and angiosarcomas compared with controls. The combined incidence of these tumors was outside the range of historical controls. These tumors are considered to be uncommon and therefore, the CARC concluded they could not be discounted. There was a split vote among Committee

members regarding adequacy of dosing. However, the majority concluded that the dosing at the highest dose was adequate and not excessive in both sexes based on increased liver weight and presence of non-neoplastic changes.

The acceptable genetic toxicology studies indicated that CGA 184927 was not mutagenic in *Salmonella typhimurium* and cultured Chinese hamster V79 lung fibroblast cells. It was not clastogenic *in vivo* and did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. The submitted *in vitro* cytogenetic assay was unacceptable. The CARC therefore, recommended that an *in vitro* cytogenetic assay be conducted to fulfill the guideline requirements. This recommendation was strengthened by the evidence from the literature that propargyl alcohol, a possible metabolite of CGA 184927, induced chromosome aberration *in vitro*.

Structurally-related compounds, haloxyfop-methyl and diclofop-methyl are hepatocarcinogens in mice while fluazifop-butyl and diclofop-methyl are non mutagens.

The Committee determined that the mechanistic studies do not support the proposed mode of action for the occurrence of prostate and ovarian tumors in rats or liver and blood vessel tumors in mice.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified clodinafop-propargyl (CGA 184927) as **"likely to be carcinogenic to humans "** by the oral route based on the occurrence of prostate tumors in male rats, ovarian tumors in female rats and liver tumors in both sexes of mice, as well as blood vessel tumors in female mice. For the quantification of human cancer risk, the Committee recommended a linear low-dose extrapolation approach based on the most potent of these tumor types. This approach is supported by possible genotoxic potential and the lack of confirmation of the mode of action of CGA 184927.

I. INTRODUCTION

On September 29, 1999, the Cancer Assessment Review Committee (CARC) met to review the carcinogenic potential of clodinafop-propargyl (CGA 184927). Dr. Yung Yang of the Toxicology Branch described the two-year chronic toxicity/carcinogenicity study in Tif: RAIf (SPF) albino rats and a life-time carcinogenicity study in Tif:MAGf (SPF) albino mice by detailing 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 dose levels tested, and presenting the weight-of-the-evidence for the carcinogenicity of clodinafop-propargyl (CGA 184927). Dr. Yang also discussed the findings of the mechanistic studies submitted by the Registrant in support of the cytochrome p-450 mediated mechanism of prostate and ovarian tumor induction in rats and the role of CGA-184927 as a peroxisome proliferator in liver tumor induction in mice.

II. BACKGROUND INFORMATION

Clodinafop-propargyl (CGA 184927) is the active ingredient of Clodinafop 2E herbicide for use on wheat. The PC Code is 125203 and the CAS number is 105511-96-4. There are no residential uses; however, there is potential for residential exposure to spray drift resulting from aerial application. Based on the use pattern, there is potential for short-term exposures (private- one field) and intermediate-term exposure (commercial- several fields) during mixing, loading, application, and post-application activities, long-term exposure is not expected to occur.

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III. EVALUATION OF CARCINOGENICITY STUDIES

1. <u>Combined Chronic Toxicity/Carcinogenicity Study with Clodinafop-propargyl in Tif:RAIF(SPF) Albino Rats</u>

<u>Reference</u>: Fankhauser, H. (1992). 24-Month Carcinogenicity and Chronic Toxicity Study in Rats. Novartis Crop Protection, Inc., Greensboro, NC. Report No. 861139; October 21, 1992. MRID 44399147 (Unpublished)

A. Experimental Design

Clodinafop-propargyl (CGA 184927)(93.7%, a.i.) was administered via diet to Tif: RAIf (SPF) albino rats (80/sex/group) at dose levels of 0, 1, 10, 300 or 750 ppm (0, 0.031, 0.32, 10.18, or 26.28 mg/kg/day for males; and 0, 0.034, 0.36, 11.31, or 29.48 mg/kg/day for females, respectively) for 24 months. At interim sacrifice, during week 53, 10 rats/sex/dose were sacrificed.

B. Discussion of Tumor Data

Treatment with CGA 184927 increased the incidence of prostate and ovarian tumors at 750 ppm. For males, an increased incidence of prostate adenoma prostate adenomas and/or carcinomas combined was seen in the high-dose group, i.e., incidence rates were: adenomas: 8/67 (12%), 9/68 (13%), 12/67 (18%), 12/68 (18%) or 19/67 (28%) and combined adenomas/carcinomas: 8/67 (12%), 9/68 (13%), 12/67 (18%), 12/68 (18%) and 20/67 (30%) in the 0, 1, 10, 300 and 750 ppm groups, respectively.

For females, an increased incidence of ovarian tubular adenomas was noted in the high-dose group, i.e., incidence rates were 2/67 (3%), 1/65 (2%), 1/70 (1%), 1/68 (1%) and 9/66 (14%) in the 0, 1, 10, 300 and 750 ppm groups, respectively.

Statistical analysis indicated that male rats had significant increases in the pair-wise comparisons of the 750 ppm dose group with the controls for prostate adenomas at $p < 0.05$ and for prostate adenomas and/or carcinomas combined at $p < 0.01$. There were also significant increasing trends in prostate adenomas and adenomas and/or carcinomas combined, both at $p < 0.01$. Female rats had a significant increase in the pair-wise comparison of the 750 ppm dose group with the controls at $p < 0.05$, for ovarian tubular adenomas. There was also a significant increasing trend at $p < 0.01$ for ovarian tubular adenomas. However, historical control data for prostate and ovarian tumors for the comparable study duration were not available for comparison.

The statistical analyses of male and female rats were based upon the Fisher's Exact test for pair-wise comparisons and the Exact test for trend. See Tables 1 and 2 for rat tumor analysis results.

Table 1. CGA 184927™ - Tif:RAIf(SPF) Albino Rat Study

Male Prostate Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results (p values)

	Dose (ppm)				
	0	1	10	300	750
Adenomas (%)	8/67 (12)	9/68 (13)	12/67 (18)	12/68 (18)	19[a]/67 (28)
p =	0.006**	0.513	0.234	0.245	0.015*
Carcinomas (%)	0/67 (0)	0/68 (0)	0/67 (0)	0/68 (0)	1[b]/67 (1)
p=	0.199	1.000	1.000	1.000	0.500
Combined (%)	8/67 (12)	9/68 (13)	12/67 (18)	12/68 (18)	20/67 (30)
p =	0.003**	0.513	0.234	0.245	0.009**

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

[a]First prostate adenoma not in an interim sacrifice animal observed at week 71, dose 750 ppm.

[b]First prostate carcinoma observed at week 106, dose 750 ppm.

Note: Interim sacrifice animals are not included in this analysis. One animal in the 300 ppm does group of the interim sacrifice group had a prostate 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 2. CGA 184927™ - Tif:RAIf(SPF) Albino Rat Study

Female Ovarian Tumor Rates[+] and Fisher's Exact Test
and Exact Trend Test Results (p values)

	Dose (ppm)				
	0	1	10	300	750
Tubular Adenomas[#] (%)	2/67 (3)	1/65 (2)	1[a]/70 (1)	1/68 (1)	9/66 (14)
p =	0.000**	0.512	0.484	0.494	0.026*

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

[#]No ovarian tubular carcinomas were observed.

[a]First ovarian tubular adenoma observed at week 87, dose 10 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no ovarian tubular adenomas 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 $p < 0.01$.

C. Non-Neoplastic Lesions

The primary target organ was the liver. Increased incidences of the following treatment-related findings were noted: hepatocyte hypertrophy in the 300 and 750 ppm groups (both sexes) and in the 10 ppm group (males only); necrosis in the 300 and 750 ppm groups (both sexes); nodular hyperplasia, fibrosis of the liver parenchyma and capsule in the 750 ppm group (both sexes) and in the 300 ppm group (males only); necrosis of hepatocytes in the 750 ppm group (both sexes); and focal hyperplasia in the 300 and 750 ppm groups (males only).

Other observations including increased incidences of hypertrophy of the thyroid follicular epithelium and medullary tubular hyperplasia in the ovary at the high-dose, and an increase in the incidence of chronic progressive nephropathy in the kidney at 300 and 750 ppm (both sexes).

D. Adequacy of the Dosing for Assessment of Carcinogenicity

There was no adverse effect on survival for either sex. All dose groups and controls had ≥55% survival at study termination. The body weight gains were comparable among all dose groups. The doses tested were considered by the CARC to be adequate and not excessive in both sexes based on increased liver weights, and histopathological changes in the liver, thyroid, ovary, and kidney.

2. Carcinogenicity Study in Mice

Reference: Fankhauser, H. (1992). 18-Month Carcinogenicity Study in Mice. Sisseln Facility, Ciba-Geigy Ltd., Switzerland for Novartis Crop Protection, Inc., Greensboro, NC 27419. Report No. 861138; July 17, 1992 MRID 44399143 (Unpublished)

A. Experimental design

Tif:MAGf (SPF) albino mice (60/sex/group) were fed CGA 184927 (93.7% a.i.) via diets at dose levels of 0, 1, 10, 100 or 250 ppm (0, 0.113, 1.10, 11.0 or 29.6 mg/kg/day for males and 0, 0.129, 1.25, 12.6 or 33.1 mg/kg/day for females, respectively) for 18 months.

B. Discussion of Tumor Data

Male mice had significantly increased incidences of hepatomas, i.e., incidence rates were 7/58 (12%), 9/52 (17%), 7/54 (13%), 10/52 (19%), or 30/53 (57%) in 0, 1, 10, 100 or 250 ppm groups, respectively. The incidence of hepatocellular carcinomas also was increased, i.e., 2/58 (3%), 4/57 (7%), 2/57 (4%), 4/57 (7%), or 8/57 (14%) in 0, 1, 10, 100, or 250 ppm groups, respectively. The combined incidences of hepatocellular adenomas/carcinomas were 9/58 (16%), 13/57 (23%), 9/57 (16%), 1/57 (25%), or 38/57 (67%) in 0, 1, 10, 100, or 250 ppm groups, respectively. Statistical analysis indicated that male mice had significant increases in the pair-wise comparisons of the 250 ppm dose group with the controls, for hepatocellular benign hepatomas and benign hepatomas and/or

carcinomas combined, all at $p < 0.01$. There were significant ($p<0.01$) increasing trends in for hepatocellular benign hepatomas, hepatocellular carcinomas ($p < 0.05$) and benign hepatomas and/or carcinomas combined, all at $p < 0.01$. The range of historical control data for benign hepatoma was 2.04-36.67%, mean 19.91%, for hepatocellular carcinoma was 0-27.12%, mean 7.79% and for combined tumors was 2.04-55.93%, mean 16.36%.

In females, the incidences of hepatomas were 0/57 (0%), 0/53 (0%), 1/56 (2%), 1/58 (2%), or 4/58 (7%) in 0, 1, 10, 100, or 250 ppm groups, respectively. Female mice had significant increasing trends in hepatocellular benign hepatomas at $p < 0.01$ and benign hepatomas and/or carcinomas combined at $p < 0.05$. However, the incidences of these tumors were not significant by pair-wise comparison with controls (7% vs 0%, p=0.06).

The statistical analyses of male mice were based upon Peto's Prevalence Test since there was statistically significant increased mortality with increasing doses of CGA 184927 in male mice. The statistical analyses of female mice were based upon the Fisher's Exact test for pair-wise comparisons and the Exact test for trend. See Tables 3 and 4 for mouse tumor analysis results.

In addition, there were slight increases in the incidence of angiosarcoma (2/60, 3%), hemangioma (2/60, 3%), and combined angiosarcoma/hemangioma (4/60, 7%) compared with concurrent controls (0%) in females at 250 ppm. The incidences of these tumors (combined) were outside the range of historical controls (0-3.75%, mean 1.25%). These tumors were not analysed statistically. However, they are considered to be uncommon, and therefore, the CARC concluded they could not be discounted.

Table 3. CGA 184927™ - Tif:MAGf(SPF) Albino Mouse Study

Male Liver Tumor Rates+ and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	1	10	100	250
Benign Hepatomas (%)	7/58 (12)	9/52 (17)	7/54 (13)	10/52 (19)	30[a]/53 (57)
p =	0.000**	0.242	0.456	0.148	0.000**
Carcinomas (%)	2/58 (3)	4[b]/57 (7)	2/57 (4)	4/57 (7)	8/57 (14)
p=	0.037*	0.294	0.478	0.279	0.072
Combined (%)	9/58 (16)	13/57 (23)	9/57 (16)	14/57 (25)	38/57 (67)
p =	0.000**	0.180	0.449	0.105	0.000**

+Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First liver benign hepatoma observed at week 77, dose 250 ppm.
[b]First liver carcinoma observed at week 59, dose 1 ppm.
Note: 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 4. CGA 184927™ - Tif:MAGf(SPF) Albino Mouse Study

Female Liver Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results (p values)

	Dose (ppm)				
	0	1	10	100	250
Benign Hepatomas (%)	0/57 (0)	0/53 (0)	1[a]/56 (2)	1/58 (2)	4/58 (7)
p =	0.005**	1.000	0.496	0.504	0.061
Carcinomas (%)	0/57 (0)	1[b]/53 (2)	0/56 (0)	0/58 (0)	0/58 (0)
p=	0.390	0.482	1.000	1.000	1.000
Combined (%)	0/57 (0)	1/53 (2)	1/56 (2)	1/58 (2)	4/58 (7)
p =	0.014*	0.482	0.496	0.504	0.061

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First liver benign hepatoma observed at week 80, dose 10 ppm.

[b]First liver carcinoma observed at week 80, dose 1 ppm.

Note: Significance of trend denoted at control.

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

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

C. Non-neoplastic Lesions

The primary target organ was the liver. The following increased incidences of treatment-related findings were noted: hepatocyte hypertrophy, Kupffer cell pigmentation and intrahepatic bile duct hyperplasia, lymphohistiocytic infiltration in the 250 ppm group (both sexes) and the 100 ppm group (females only), hepatocyte necrosis in the 100 and 250 ppm groups (males only), and necrosis in the 250 ppm group (females only).

D. Adequacy of Dosing for Assessment of Carcinogenicity

The doses tested were considered to be adequate and not excessive in both sexes based on decreases in body weight gain (11%) in males, increased liver enzyme activity and liver weights, and histopathological changes in the liver in both sexes. The severity of these lesions and their contribution to male mortality was unclear. Females at the low dose had higher mortality than at the high dose and death in high dose females occurred late during the study period. Despite high mortality among high-dose males, a sufficient number of males survived at study termination. There was a split vote among CARC members about dose adequacy. Three CARC members thought the high dose in males was excessive. The vote for females was yes: 8, vs no: 5 on whether the high dose was adequate.

IV. TOXICOLOGY

1. Metabolism

In a metabolism study (MRID 44399159), two ^{14}C labeled variants of CGA 184927 (one labeled on the 2 pyridil carbon and the other universally labeled on the phenyl ring, purity >98%) were administered to groups of five male Tif:RAI f (SPF) rats, approximately 7 weeks of age (weighing about 200 g at time of dosing) by gavage at concentrations of 25.2 mg/kg ([2-^{14}C]pyridil) and 24.6 mg/kg ([U-^{14}C]phenyl). For dosing, the test material was dissolved in polyethylene glycol 200/ethanol/water (7/5/2) and 0.7 ml/rat was administered via gavage. Individual urine and fecal samples were collected at 24 hour intervals for 7 days post-dosing, after which animals were sacrificed by cervical dislocation. Samples of liver, kidney and fat, and the remaining carcass, were retained for residue analysis.

After a single oral dose of about 25 mg/kg b.w, an approximately 70.0% of the dose was absorbed from the intestinal tract. Recovery of the radiolabel ranged from 97-105%. In [U-^{14}C]phenyl CGA 184927 treated rats, the excretion of radioactivity in the urine and feces was 48.4% and 22.3% of the administered dose (AD), respectively. Of this, only 15.4% (urine) and 11.2% (feces) of the AD had been eliminated within the first 24 hours. Carcass and tissues contained 25.1% of the AD. Residues in fat, liver, kidney and carcass were 3.8%, 0.8%, 0.2% and 20.3% of the AD, respectively. In [2-^{14}C]pyridil CGA 184927 treated rats, the excretion of radioactivity in the urine and feces was 51.1% and 23.6% of the AD, respectively. Of this, only 14.8% (urine) and 13.3% (feces) of the AD had been

eliminated within the first 24 hours. Carcass and tissues contained 22.9% of the AD. Residues in fat, liver, kidney and carcass were 3.9%, 0.9%, 0.2% and 17.9% of the AD, respectively. These results indicate that the CGA 184927 is excreted slowly and most of the residual radioactivity remaining in the carcass (18%-20%). Results from both labelling studies were almost identical indicating that the diaryl ether bond of CGA 184927 is not cleaved to any significant extent.

Metabolic profiles were determined by thin layer chromatography (TLC), liquid chromatography (LC), high performance liquid chromatography (HPLC) and high voltage electrophoresis (HVE). Metabolite patterns (urine, fecal and tissue) for [U-^{14}C]phenyl-, and [2-^{14}C]pyridil-labelled CGA 184927 were almost identical. In the urine, the major metabolite (fraction U7) was determined to be (R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propionic acid, reference material CGA 193469, accounting for 36.7% to 39.1% of the AD. In addition, seven unidentified metabolites were isolated, ranging from 0.1% to 5.2% of the AD. Metabolite fraction U3 hydrolysed to yield fraction U7 (i.e., CGA 193469), when treated with NaOH or HCl. Unchanged CGA 184927 was not identified. In the feces, the major metabolite (fraction F*7) corresponded to the urinary metabolite U7 (CGA 193469), accounting for 15.7% to 16.9% of the AD. Metabolite fraction F*8 was determined to be unchanged CGA 184927, accounting for 0.4% to 1.7% of the AD. Six unidentified metabolites were isolated, ranging from 0.3% to 1.4% of the AD. In the fat, all metabolites were reportedly acylglycerides, the majority of which were hybrid di- and triacylglycerides, i.e., approximately 3.5% and 17.0% of the AD, respectively.

In the liver, kidney and carcass, the metabolic pattern reflected the transformations seen in excreta and fat.

2. Mutagenicity

The submitted genetic toxicology studies indicate that CGA 184927 is not mutagenic in bacteria (*Salmonella typhimurium*) or cultured mammalian cells (Chinese hamster V79 lung fibroblasts). There is also no evidence of clastogenicity *in vivo*. Similarly, CGA 184927 did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. However, the submitted studies do not satisfy the 1991 mutagenicity guideline requirements since the *in vitro* cytogenetic assay has been classified as unacceptable. It is recommended, therefore, that an *in vitro* cytogenetic assay be conducted to fulfill guideline requirements. This recommendation is strengthened by the evidence from the literature that propargyl alcohol, a possible metabolite of CGA 184927, induced chromosome aberration *in vitro* (Blakey, et al., 1994). Summaries of the acceptable studies are presented below:

A. Gene Mutations

a) *S. typhimurium* mammalian microsome gene mutation assay: Independently performed trials were negative up to insoluble doses (≥313 μg/plate) with or without S9 activation. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (MRID No. 44399153).

b) *In vitro* mammalian cell forward gene mutation assay in Chinese hamster V79 cells: Independently performed trials were negative up to cytotoxic concentrations (500 μg/mL without S9 activation and ≈41-50 μg/mL in the presence of S9 activation). The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a mammalian cell gene mutation assay (MRID No. 44399152).

B. Chromosome Aberrations

In vivo micronucleus assay: The test was negative in male and female NMRI mice administered single doses of 1667 or 5000 mg/kg by oral gavage. Lethality was seen in the high-dose group. There was no evidence of bone marrow cytotoxicity. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for an *in vivo* cytogenetic assay (MRID No. 44399151).

C. Other Mutagenic Mechanisms

In vitro UDS assay in primary rat hepatocytes: Independently performed trials were negative up to insoluble doses (≥4000 μg/mL). The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a UDS assay (MRID No. 44399156).

D. Additional Information

Blakey et al. (1994) indicated that propargyl alcohol induced chromosomal aberration in CHO cells *in vitro* with and without metabolic activation, while CGA 184927 did not induce reverse mutation detectable with the *Salmonella*/mammalian microsome assay. The formation of propargyl alcohol from CGA 184927 requires ester hydrolysis and under the conditions of the mutagenicity assays ester hydrolysis may not occur, thus giving the false impression that CGA 184927 is non mutagenic.

3. **Structure Activity Relationship**

Two of the four structural analogs (i.e., haloxyfop-methyl and diclofop-methyl) were found to induce liver tumors in mice. The diphenyl ether like structure in the molecule may be responsible for the carcinogenic potential of these compounds. Fluazifop-butyl and diclofop-methyl are not mutagenic. The mutagenicity data on other compounds were not available.

Compound	Structure	Carcinogenic Effect
Clodinafop-propargyl CAS 105511-96-4 PC 125203	F, O, O, O, CH, H, Cl, N, O, CH_3	- Prostate and ovarian tubular tumors in rats. - Liver tumors in mice

Haloxyfop-Methyl CAS 69806-40-2 PC 125201		- Classification: B2 - Liver tumor (adenoma and carcinomas). - B6C3F1 mice. - Mutagenicity data not available.
Fluazifop-Butyl CAS 69806-50-4 PC 122805		- No evidence of increased liver tumors; however, the dose levels may be inadequate. - Not mutagenic.
Diclofop-Methyl (Hoelon) CAS 51338-27-3 PC 110902		- Classification: Group C; possible human carcinogen - Hepatocellular adenoma and/or carcinomas - NMRKf (SPF) mice. - Not mutagenic.
Clofop-Isobutyl CAS 51337-71-4		- No data are available. - The chemical has been voluntarily canceled.

Peroxisome proliferation studies on related compounds were not available.

4. Subchronic and Chronic Toxicity

A. Subchronic Toxicity

a) In a subchronic toxicity study in rats (MRID# 44399132), CGA 184927 (93.7%, a.i.) was administered to Tif: RAIf (SPF) albino rats (20/sex/group) via diet at dose levels of 0, 2, 15, 120, or 1000 ppm (0, 0.13, 0.92, 8.24, or 70.0 mg/kg/day for males; and 0, 0.13, 0.94, 8.24, or 71.1 mg/kg/day for females, respectively) for a period of 92 to 94 days.

Significant findings included decreases in mean bodyweight and mean absolute and relative thymus weight (males only), and increases in mean absolute and relative liver weights. Histopathological findings considered to be treatment-related were hepatocytic hypertrophy seen in both sexes, and thymic atrophy observed in males only. After a 28-day recovery period, it was demonstrated that the treatment-related findings were reversible.

The LOAEL is 120 ppm (8.24 mg/kg/day) for males and 1000 ppm (71.1 mg/kg/day) for females, based on increased liver weights and enzyme activity in males at 120 ppm and liver hypertrophy in both sexes at 1000 ppm. The NOAEL is 15 ppm (0.92 mg/kg/day) for males, and 120 ppm (8.24 mg/kg/day) for females.

b) In a 28-day oral (gavage) toxicity study in rats (MRID# 44399130), CGA 184927 (93.7% a.i.) suspended in water containing 0.5% carboxymethylcellulose and 0.1% Tween 80 was administered to Tif:RAIf (SPF) rats at dosage levels of 0, 5, 40 and 200 mg/kg/day for 28 consecutive days.

Mortality was excessive in males at the high dose level (all rats in this group died prior to term). One female in the high dose group died prior to term. The primary target organ for toxicity was the liver, where findings such as liver enlargement, increased liver weight and hepatocellular hypertrophy were observed at all doses in males and at 40 and 200 mg/kg/day in females.

The systemic toxicity LOAEL is 5 mg/kg/day based on adverse effects in the liver. A definitive NOAEL is not identified in this study for either sex.

c) In a subchronic toxicity study in dogs (MRID# 44399139), CGA 184927 was administered to beagle dogs (4/sex/group). The test diets contained technical CGA 184927, purity 84.3% administered during study weeks 1 and 2, and purity 93.7% administered for the remainder of the study, at dietary concentrations of 0, 1/1000/500 (days 1-54/55-66/67-90), 10, 50 or 200 ppm (0.038/34.07/16.43, 0.346, 1.73, 7.91 mg/kg/day for males and 0.036/32.29/16.86, 0.390, 1.89 and 7.16 mg/kg/day for females, respectively) for a period of 90 days.

In the 50 ppm group (males only) and the 200 ppm group (females only) pustule formation was noted in the inguinal/abdominal areas. At 1000 ppm, more extensive skin lesions were observed, characterized as general erythema of the skin, pustule formation, conjunctivitis, purulent areas, alopecia and encrustation. In addition, tremors (8 days after dosing but were transient), decreased activity (persisted until necropsy), increased incidence of diarrhea and decreased bodyweight and food consumption were noted during the 1000 ppm dosing period. Decreased RBC count, Hgb and HCT were noted at 1/1000/500 ppm group. At 13 weeks, increased alkaline phosphatase (both sexes), alanine aminotransferase (females only) and aspartate aminotransferase (females only) were noted in the 1/1000/500 ppm (both sexes) group. Mean absolute and relative liver and kidney weights were increased in the 1/1000/500 ppm group, males only. However, these later findings were statistically non-significant and were not supported by histopathological changes in these organs. Histopathological changes consisting of acute hemorrhagic to purulent pneumonia, duodenal ulceration and acute focal ulcerative dermatitis, considered to be treatment-related were only observed in the male dog sacrificed in extremis on study day 71 (1/1000/500 ppm group). The only other finding considered to be treatment-related was vacuolated cell foci in the zona fasciculata of the adrenal cortex in all animals of the 1/1000/500 ppm group. This change was not observed in any other animal at any other dose level tested.

The LOAEL is 50 ppm (1.73 mg/kg/day) for males and 200 ppm (7.16 mg/kg/day) for females, based on occurrence of skin lesions. The NOAEL is 10 ppm for males (0.346 mg/kg/day), and 50 ppm for

females (1.89 mg/kg/day).

B. Chronic Toxicity

Rat

In a combined chronic toxicity/carcinogenicity study (MRID# 44399147), CGA 184927 (93.7% a.i.) was administered in diet to Tif: RAIf (SPF) albino rats (80/sex/group) for a period of 24 months. The test diets contained technical CGA 184927 at dietary concentrations of 0, 1, 10, 300 or 750 ppm (0, 0.031, 0.32, 10.18, 26.28 mg/kg/day for males; and 0, 0.034, 0.36, 11.31, 29.48 mg/kg/day for females, respectively). At interim sacrifice, week 53, 10 rats/sex/dose were sacrificed.

An increase in liver enzyme levels was noted in both sexes at ≥300 ppm. At interim and final sacrifices, absolute and/or relative liver and kidney weights increased in one or both sexes at ≥300 ppm. Necropsy revealed increased incidence of enlarged, or mottled liver in males at ≥300 ppm and in females at 750 ppm. In addition, there was a dose-related increase in the incidence of microscopic changes in the liver including hepatocytic hypertrophy in males at ≥10 ppm as well as focal or nodular hyperplasia and fibrosis in one or both sexes at ≥300 ppm. At 750 ppm, one of 80 males developed hepatocarcinoma. Hypertrophy of the follicular epithelium in the thyroid was noted in females at 750 ppm. Kidney changes noted in both sexes consisted of increased incidence of chronic progressive nephropathy and tubular pigmentation at ≥10 ppm. An increased incidence of ovarian medullary tubular hyperplasia was noted at ≥300 ppm.

The LOAEL for systemic toxicity is 10 ppm (0.32 and 0.36 mg/kg/day in males and females, respectively) based on hepatocytic hypertrophy, chronic progressive nephropathy and tubular pigmentation. The systemic NOAEL is 1 ppm (0.031 and 0.034 mg/kg/day in males and females, respectively).

Mouse

In an 18-month carcinogenicity study (MRID# 44399143), Tif:MAGf (SPF) albino mice (60/sex/group) were fed diets containing 0, 1, 10, 100 or 250 ppm (0, 0.113, 1.10, 11.0 or 29.6 mg/kg bw/day for males and 0, 0.129, 1.25, 12.6 and 33.1 mg/kg bw/day for females, respectively) CGA 184927 (93.7% a.i.).

At 250 ppm, among males, there was increased mortality (12/60; 20%) during the last month of the study; a high proportion of them (38/60; 63%) developed hepatocellular tumors. The mean final body weight (5-6%) and mean overall body weight gain (11%) were lower than controls in males only. At ≥100 ppm, there were increases in liver enzyme activity and liver weights in both sexes. At necropsy, an increased incidence of enlarged livers and liver nodules/masses were noted in males at 100 ppm and in males and females at 250 ppm. Histopathology revealed non-neoplastic changes including hepatocytic hypertrophy, kupffer cell pigmentation and intrahepatic bile duct hyperplasia in males at 100 ppm and in males and females at 250 ppm; hepatocytic necrosis in males at 100 and 250 ppm and

necrosis in females at 250 ppm. In addition, an increased incidence of pre-neoplastic foci was noted at 100 and 250 ppm in males only; an increase in the severity of thymic atrophy was seen in both sexes.

The LOAEL for systemic toxicity is 11.0 and 12.6 mg/kg/day for males and females, respectively, based on increases in liver enzyme activity and liver weights. The NOAEL was estimated to be 1.10 and 1.25 mg/kg/day for males and females, respectively.

Dog

In a 52-week chronic toxicity study (MRID# 44399142), beagle dogs (4/sex/group) were fed diets containing 0, 10, 100 or 500 ppm (0, 0.32, 3.38 or 15.2 mg/kg/day for males and 0, 0.32, 3.37 or 16.7 mg/kg/day for females, respectively) CGA 184927 (93.7% a.i.).

There was no effect on the survival of animals. At 500 ppm, skin lesions (pustules, crusts, scales, erythema, increased severity of alopecia, fissures, reddened sclera and ocular exudates), clinical signs of toxicity (decreased activity, paddling movements, abnormal gait or uncoordinated movements, pallor, dyspnea, diarrhea), and decreased body weight gain (-12% and -35% in males and females, respectively) were noted. In addition, hematology revealed decreased platelet count and increased thromboplastin time in females and reduced iron concentration in males.

The LOAEL is 500 ppm (15.2 and 16.7 mg/kg/day for males and females, respectively), based on occurrence of skin lesions, clinical signs and reduced body weight gain and food consumption. The NOAEL was determined to be 100 ppm (3.38 and 3.37 mg/kg/day for males and females, respectively).

5. Mechanistic Studies

The Registrant proposed that clodinafop-propargyl (CGA 184927) acts as a peroxisome proliferator and is directly involved with the onset of liver carcinogenesis in the rodent. Peroxisome proliferation is a transcription-mediated process that involves activation by the peroxisome proliferator of a nuclear receptor in rodent liver called the peroxisome proliferator-activated receptor (PPARα), a member of the steroid hormone receptor superfamily. Upon activation by peroxisomal proliferators, PPARα forms a heterodimer with the retinoid-X-receptor. This dimer binds to peroxisome proliferator response elements (PPRE) in the promoter region of target genes known to be regulated by PPARα. PPARα induces mitogenesis and cell proliferation which can lead to the formation of hepatocellular tumors. Oxidative stress appears to play a significant role in this increased cell proliferation. It triggers the release of Tumor Necrosis Factor (TNFα) by Kupffer cells, which in turn acts as a potent mitogen in hepatocytes.

The effects caused by peroxisome proliferators in endocrine organs are likely to be a consequence of altered steroid hormone metabolism. The following studies discuss the role of CGA 184927 as a peroxisome proliferator and its role in induction of peroxisomal and microsomal enzymes, alteration

in steroid metabolism resulting in tumor formation.

A) Waechter, F. (1991). The Effect Of CGA 184927 On Selected Biochemical Parameters In The Rat Liver Following Subchronic Administration. MRID NUMBER: 44399137

In a 3-month subchronic study, liver samples were obtained from the control (10 rats/sex/group), 2, 15, 120 and 1000 ppm groups (6 rats/sex/group) and subcellular fractions were prepared to measure various biochemical parameters. These included protein content (supernatant, microsomal and cytosolic), microsomal cytochrome P-450, microsomal ethoxycoumarin O-deethylase, microsomal metabolism of R-warfarin, microsomal hydroxylation of lauric acid, microsomal epoxide hydrolase, microsomal UDP-glucuronosyltransferase, and cytosolic glutathione S-transferase, as well as beta-oxidation of [1-^{14}C]palmitoyl-CoA. The microsomal fractions were also subjected to SDS PAGE and immunoblot analysis.

At 14 weeks, there was an increase in all the above parameters at one or more dose levels with the exception of decrease in glutathione S-transferase in males at 1000 ppm.

At 18 weeks, there was a statistically significant but slight increase in microsomal protein, cytochrome P-450 and fatty acyl-CoA beta-oxidation, seen in the high-dose group, males only. However, these data indicate partial recovery toward normal levels. All other parameters measured had recovered to normal control levels.

Immunoblot analyses reported an induction of cytochrome p-452 in both sexes at 1000 ppm and in males at 2, 15, and 120 ppm. Increased enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase (peroxisomal bifunctional enzyme) was noted in both sexes at 1000 ppm. The major phenobarbital-inducible cytochrome P-450 isozymes b and e as well as the major polycyclic aromatic hydrocarbon-inducible cytochrome P-450 isozymes c and d were not induced.

The effects of CGA 184927 on selected liver enzymes in the rat were similar to the effects seen after subchronic treatment with known peroxisome proliferators (hypolipidemic compounds, phenoxyacetic acid derivatives). Hence, CGA 184927 was considered most likely to be a peroxisome proliferator in the rat liver.

B) Staubli, W. and Bentley, P. (1989). Electron Microscopical Study, PS 3.3 Cell Biology, dated April 25, 1989. MRID NUMBER: Not assigned

In a subchronic study at necropsy, liver samples were taken from CGA 184927 treated animals at 14 weeks (2/sex/dose from the control and high-dose groups and during recovery period, at 18 weeks (3/sex/dose from the control and 1000 ppm dose groups). These samples were processed and examined by electron microscopy.

At 14 weeks, an increase in the number and size of peroxisomes with matrical inclusion bodies was noted in the 1000 ppm group, males only. At 18 weeks, the number and size of peroxisomes in the

1000 ppm group, both sexes, were comparable to the control group. However, an increased number of matrical granules was observed in hepatic mitochondria indicating that these mitochondrial changes were not fully reversible during the 4-week recovery phase.

The results of electron microscopic examination of liver tissue support the conclusion that CGA 184927 is a peroxisome proliferator in the rat liver.

C) Trendelenburg, C. (1999). Effects on selected plasma concentrations and biochemical parameters in the liver upon subchronic administration to male adult rats. MRID 44767401.

CGA 184927 (94.3% a.i.) was administered to 10 male Tif:RAIf (SPF) rats/dose at dietary dose of 0 or 750 ppm (0 or 53.5 mg/kg/day) for 14 days. Treatment with CGA 194927 caused no clinical signs of toxicity. Rats in the 750 ppm exhibited decreased feed consumption relative to the control group during the first 4 days of dosing which resulted in decreased body weights which persisted for the duration of the study. Although absolute and relative liver weights increased (134% and 139% of control, respectively). There were no changes in organ weights of endocrine and sex hormone dependent organs.

After 14 days of treatment, there was significant increase in liver peroxisomal fatty acid β-oxidation activity (807% of control) as well as a significant increase in liver microsomal cytochrome P450 isoenzymes CYP4A1/A3 and CYP4A2 (242% and 618% of control, respectively), thus supporting the proposed mechanism of peroxisomal proliferation in the rat liver by CGA 184927.

The treatment with CGA 184927 significantly ($p < 0.05$) decreased the total liver microsomal testosterone oxidation rate to 34% of control. Hydroxylation rates at positions 2α and 16α decreased to 19% and 20% of control (significantly; $p < 0.05$), respectively, and the oxidation rate to androstenedione was decreased to 43% of control. The reduced hydroxylation rates at positions 2α and 16α and the conversion of testosterone to androstenedione indicated depletion of the male specific cytochrome P450 isoenzyme CYP2C11. Decrease in testosterone hydroxylation rates at positions 2 β, 6α, 6 β and 7α (63, 84, 70 and 50%, respectively) were indicative of partial depletion of cytochrome P450 isoenzymes of subfamilies CYP2A and CYP3A. The treatment resulted in significant increases in hepatic aromatase activity ($p < 0.05$; 169%) and plasma estradiol concentration (not significant; 179% and 140% (CYP19A1) for 3 and 14 days, respectively) compared to control. The total and free plasma testosterone concentrations were not effected by treatment with CGA-184927. Plasma 5α-dihydrotestosterone level increased (not significantly; 198% of control) after 14 days of treatment. No change in plasma concentrations of prolactin, follicular stimulating and luteinizing hormone were noted.

These findings indicate that in the male rat, CGA 184927 acts as a peroxisomal proliferating agent and alters monooxygenase activity in subfamilies of cytochrome P450 which are known to be involved in the synthesis or catabolism of steroid hormones.

D) Bieri, F. (1991). The Effect of CGA193469, The Free Acid Derivative of CGA 184927, on Peroxisomal Beta-Oxidation in Primary Cultures of Rat, Mouse, Marmoset and Guinea P ig Hepatocytes. MRID NUMBER: 44399157

Following oral administration, CGA 184927 is rapidly hydrolyzed to CGA 193469 (acid derivative) and propargyl alcohol. The effect of CGA 193469 on peroxisomal beta-oxidation was investigated in rat, mouse, marmoset and guinea pig hepatocytes cultured in vitro.

CGA 184927-induced cytotoxicity through propargyl alcohol released following its enzymatic hydrolysis. In cultured hepatocytes from all species, CGA 193469 induced in the cultured rat and mouse hepatocytes the effect characteristically produced by other peroxisomal proliferators. In rat hepatocytes, it induced-peroxisome beta-oxidation in a dose-dependent manner. This enzyme activity was marginal in the guinea pig hepatocytes and was non-measurable in marmoset hepatocytes.

The results of this study suggest that guinea pig and primates are less susceptible to the liver effects by this class of compounds.

E) Guyomard, C. (1992). Effects of CGA 193469, the acid derivative of CGA 184927, on the peroxisomal beta-oxidation in human hepatocytes. MRID NUMBER: 44399158

Monolayer cultures of human lymphocytes were grown in a medium containing CGA 193469 (at concentrations of 0, 0.1, 1, 10 or 100 μM in 0.1% DMSO), benzafibric acid (a reference material with a strong peroxisomal beta-oxidation inducing activity in vivo and in vitro) or solvent control and incubated for 72 hours. After 24, 48 and 72 hours incubation, there were no alterations in morphology, nor in intracellular LDH levels after incubation with CGA 193469, or benzafibric acid, at any concentration tested. The protein contents were unaltered. An increase (123%) in peroxisome beta-oxidation was noted in hepatocytes incubated with CGA 193469 for 72 hours at 100 μM. However, this finding was not statistically significant, and in the absence of a dose-response relationship was not considered to be a treatment-related finding. Treatment with CGA 193469 or bezafibric acid, at all concentrations tested, was not cytotoxic to human hepatocytes, in vitro.

Under the conditions of this study, neither CGA 193469 nor bezafibric acid induced peroxisomal beta-oxidation in human hepatocytes, in vitro. However, in the absence of a known concurrent human positive control to validate the test system, i.e., a substance known to elicit peroxisomal beta-oxidation in human hepatocytes, this cannot be definitely concluded.

F) Roberts R. (1999). CGA-193469 (free acid derivative of CGA-184927) peroxisome proliferators: species difference in the regulation of gene expression (MRID 44923101).

Promoter-reporter gene assays were employed to determine the ability of CGA 193469, the free acid derivative of clodinafop-propargyl (CGA 184927), to activate the rodent and human acyl CoA oxidase (ACO) gene promoters. The data show that CGA 183469 is able to activate the rodent ACO gene promoter-reporter construct as expected for a rodent peroxisome proliferator. In contrast, CGA 193469 was unable to activate the human ACO promoter-reporter construct.

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

1. Carcinogenicity:

- **The CARC concluded that CGA-184927 was carcinogenic in male and female rats** because: 1) Males had significant differences in the pair-wise comparisons of the 750 ppm dose group with the controls for prostate gland adenomas (19/67, 28% vs 8/67, 12%; $p < 0.05$) and for combined adenomas/ carcinomas (20/67, 30% vs 8/67, 12%; $p<0.01$). There were significant ($p < 0.01$) increasing trends for prostate adenomas and combined adenomas/carcinomas; 2) Females had a significant difference in the pair-wise comparison of the 750 ppm dose group with the controls (9/66, 14% vs 2/67, 3%; $p < 0.05$), for ovarian tubular adenomas. There was also a significant ($p< 0.01$) increasing trend for ovarian tumors. Historical control data for the comparable study duration were not available. The dosing at the highest dose was considered to be adequate and not excessive in both sexes based on increased liver weights, and histopathological changes in the liver (hypertrophy, necrosis, focal hyperplasia, fibrosis of parenchyma), hypertrophy of the thyroid follicular epithelium and medullary tubular hyperplasia in the ovary, as well as chronic progressive nephropathy of the kidney. The increased incidence of ovarian adenomas at the high-dose was supported by the increased incidence of medullary tubular hyperplasia in the ovary. The CARC concluded that the prostate and ovarian tumors in rats were treatment-related.

- **CGA-194927 was also carcinogenic to mice because**: 1) Males had significant differences in the pair-wise comparisons of the 250 ppm dose group with the controls, for hepatomas (30/53, 57% vs 7/58, 12%; $p<0.01$) and combined hepatomas/carcinomas (38/57, 67% vs 9/58, 16%; $p<0.01$). The increased incidence of carcinomas at 250 ppm (8/57, 14% vs 2/58, 3%) was considered by the CARC to be biologically significant. The incidences of hepatomas and carcinomas in males were outside the historical control range (hepatomas: 2.04%-36.67%; mean: 19.91%; carcinomas: 0%-27.12%; mean: 7.79%). There were also significant increasing trends for hepatomas ($p<0.01$), carcinomas ($p<0.05$), and combined hepatomas/ carcinomas ($p<0.01$); 2) The females had significant increasing trends for hepatomas ($p < 0.01$) and combined hepatomas/carcinomas ($p< 0.05$). However, liver tumors in females were not supportive of that in males because of lack of dose-response, absence of carcinomas and the incidences of adenomas (7%) and combined adenomas/carcinomas (7%) were within the historical control range (adenomas: 0%-16.25% and carcinomas: 0%-10%). The incidences of these tumors were not significant by the pair-wise comparison; 3) Although there was a borderline increase in hemangiomas and angiosarcomas (7% vs 0%) among high-dose females, the combined incidence of these tumors was outside the historical control range (0-3.75%, mean: 1.25%). These tumors are uncommon and therefore, can not be discounted; 4) There was a split vote among the committee members regarding adequacy of dosing. The statistically

significance of increased mortality among high-dose males was questionable due to variable mortality at other dose levels. Low dose females had higher mortality than high dose females and deaths in high dose females occurred late during study period. The majority of the Committee members considered dosing at the highest dose to be adequate in both sexes and not excessive based on increased liver enzyme activity and liver weights, and histopathological changes in the liver (although the severity of the lesions and their contribution to mortality was unclear). Based on the weight-of-the-evidence, the liver tumors in male and female mice were considered by the CARC to be treatment-related.

2. Mutagenicity

- The submitted genetic toxicology studies indicate that CGA 184927 is not mutagenic in bacteria (*Salmonella typhimurium*) or cultured mammalian cells (Chinese hamster V79 lung fibroblasts). There is also no evidence of clastogenicity *in vivo*. Similarly, CGA 184927 did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. However, the submitted studies do not satisfy the 1991 mutagenicity guideline requirements since the *in vitro* cytogenetic assay has been classified as unacceptable. It is recommended, therefore, that an *in vitro* cytogenetic assay be conducted to fulfill the guideline requirements. This recommendation is strengthened by the evidence from the literature that propargyl alcohol, a possible metabolite of CGA 184927, induced chromosome aberration *in vitro* (Blakey, et al., 1994).

3. Structure Activity Relationship

- Two of the four structural analogs of clodinafop-propargyl, haloxyfop-methyl and diclofop-methyl were found to induce liver tumors in mice. Both fluazifop-butyl and diclofop-methyl are non mutagens. Mutagenicity data on other compounds was unavailable.

4. Mode of Action

- On September 27, 1999, the **Mechanism of Toxicity Assessment Review Committee (MTARC)** met to evaluate the mechanistic studies submitted by the Registrant. The **Registrant** contended that CGA-184927 acts as a rodent-specific peroxisome proliferator which manifests itself in the induction of peroxisomal and microsomal enzymes and alteration of steroid hormone metabolism resulting in tumor formation.

  The **MTARC** determined that the submitted studies do not support the proposed mode of action of peroxisome proliferation as the mechanism of liver carcinogenicity for clodinafop-propargyl (CGA 184927) based on the following reasons: (1) the submitted data were based on rat studies; however, the liver tumors were observed in mice only which raises an uncertainty about the peroxisome proliferation mechanism of

tumorigenesis in mice; (2) the structure activity relationship data indicated that there may be other possible mechanisms which may contribute to the liver carcinogenesis.

The CARC concluded that peroxisome proliferation may be one of the mechanisms in inducing cytochrome p-450 enzymes that are involved in altering the steroid metabolism but the Registrant did not provide information to support their hypothesis. However, based on the pathological changes seen (hyperplasia and hypertrophy of hepatocytes), other mechanisms are plausible. Also, while the mutagenicity data indicate that CGA 184927 is not mutagenic in bacteria or cultured mammalian cells, propargyl alcohol and propargyl aldehyde (a metabolite of propargyl alcohol) are known mutagens. The Committee recognizes that the peroxisome proliferators can act as liver tumor promoters and that there are quantitative differences in the degree of peroxisome proliferation between rodents, guinea pigs, marmosets and humans. Although the mechanistic data in rats fulfill the criteria for peroxisome proliferation, the studies were conducted in rats while liver tumors were observed in mice only. The peroxisome proliferation study was not conducted in mice. The lack of liver tumors in rats raises uncertainty regarding the role of peroxisome proliferation in mouse liver tumorigenesis. No dose-related association of peroxisome proliferating activity with increase in liver tumors in mice was demonstrated. **The CARC, therefore, concluded that these studies do not support the proposed mode of action for the occurrence of prostate and ovarian tumors in rats or liver and blood vessel tumors in mice.**

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified CGA-184927 as **"likely to be carcinogenic to humans"** by the oral route based on the following weight-of-the-evidence considerations:

1. Increased incidences of prostate tumors in male rats, ovarian adenomas in female rats and liver tumors in male and female mice and blood vessel tumors in female mice.

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

3. Structurally related compounds, haloxyfop-methyl and diclofop-methyl are hepatocarcinogens in mice. Both fluazifop-butyl and diclofop-methyl are non mutagens.

VIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose extrapolation approach based on most potent tumors for human cancer risk assessment. This approach is supported by possible genotoxic potential and the lack of confirmation of the mode of action of CGA 184927.

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