Attachment 1

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: September 21, 1999

MEMORANDUM

SUBJECT: ***Clodinafop-propargyl (CGA 184927)***: Assessment of Mode of Action on Liver Carcinogenicity

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And
Diana Locke
Executive Secretary, Common Mechanism SARC
Health Effects Division (7509C)

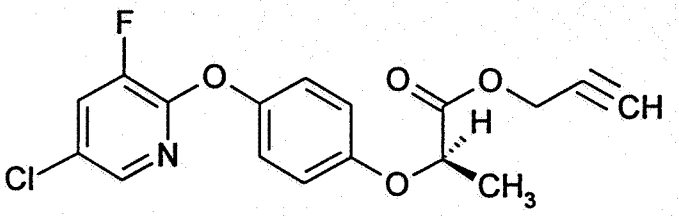
PC Code: 125203

Attached is a proposed assessment of mode of action on liver carcinogenicity of clodinafop-propargyl (CGA 184927).

An Assessment of Mode of Action on Liver Carcinogenicity of Clodinafop-propargyl (CGA 184927)

I. 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.



The Registrant has submitted studies in an effort to establish that clodinafop-propargyl is a peroxisome proliferator. The Registrant requested that the Agency should apply the proposed mode of action of proxisome proliferator as described below for conducting risk assessment on liver carcinogenicity of this chemical.

II. Evaluation of Carcinogenicity Studies

1. 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 Report No. 861138; July 17, 1992 MRID NUMBER: 44399143 (Unpublished)

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.

The neoplastic changes consisted of dose-related increased incidence of hepatomas in males (11/60 [18%] and 30/60 [50%] at 100 ppm and 250 ppm, respectively) and in females (4/60 [7%]) at 250 ppm; hepatocellular carcinomas in males (8/60 [13%]) at 250 ppm and combined incidences of hepatocellular tumors in males (15/60[25%]and 38/60[63%]at 100 ppm and 250 ppm, respectively), and in females (4/60[7%])at 250 ppm.

Statistical analysis indicated that male mice had significant increasing trends, and significant differences 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 was also a significant increasing trend in hepatocellular carcinomas at $p < 0.05$

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Female mice had significant increasing trends in hepatocellular benign hepatomas at $p < 0.01$ and benign hepatomas and/or carcinomas combined at $p < 0.05$.

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

Reference: 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 NUMBER: 44399147 (Unpublished)

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.

Treatment with CGA 184927 increased the incidence of prostate and ovarian tumors in rats at 750 ppm. For males, an increased incidence of prostate adenoma was seen in the high-dose group, i.e., incidence rates were 8/80 (10.0%), 9/80 (11.25%), 12/80 (15.0%), 13/80 (16.25%) and 19/80 (23.75%) in the 0, 1, 10, 300 and 750 ppm groups, respectively. In addition, one of 80 males developed hepatocarcinoma at 750 ppm.

For females, an increased incidence of tubular adenomas of the ovary was noted in the high-dose group, i.e., incidence rates of 2/80 (2.5%), 1/80 (1.25%), 1/80 (1.25%), 1/80 (1.25%) and 9/80 (11.25%) for the 0, 1, 10, 300 and 750 ppm groups, respectively. The chemical was administered at a dose sufficient to test its carcinogenic potential.

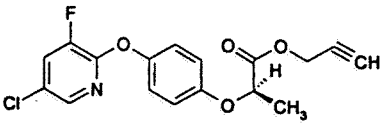
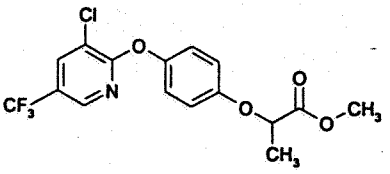
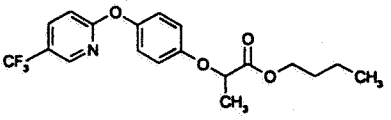
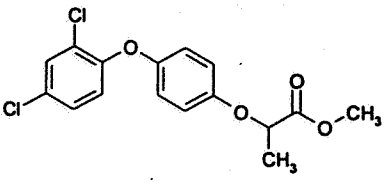
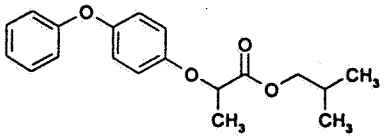
Statistical analysis indicated that male rats had significant increasing trends in prostate adenomas and adenomas and/or carcinomas combined, both at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 750 ppm dose group with the controls for prostate adenomas at $p < 0.05$ and for prostate adenomas and/or carcinomas combined at $p < 0.01$.

Female rats had a significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 750 ppm dose group with the controls at $p < 0.05$, for ovarian tubular adenomas.

3. Mutagenicity

The acceptable 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 acceptable studies do not satisfy the 1991 mutagenicity guideline requirements since the submitted *in vitro* cytogenetic assay has been classified as unacceptable.

4. Structure Activity Relationship

Compound	Structure	Carcinogenic Effect
Clodinafop-propargyl CAS 105511-96-4 PC 125203		- 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.
Fluazifop-Butyl CAS 69806-50-4 PC 122805		- No evidence of increased liver tumors; however, the dose levels may be inadequate.
Diclofop-Methyl (Hoelon) CAS 51338-27-3 PC 110902		- Classification: Group C; possible humna carcinogen - Hepatocellular adenoma and/or carcinomas - NMRKf (SPF) mice.
Clofop-Isobutyl CAS 51337-71-4		- No data is available. - The chemical has been voluntarily canceled.

III. Proposed Mode of Action of Carcinogenicity

J. Doull et al. (1999) stated that there is strong evidence and scientific consensus that, in rodents, peroxisome proliferation is directly associated with the onset of liver cancer. 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). The critical role of PPARα in peroxisomal proliferation and carcinogenicity in mice is clearly established by the lack of either response in mice genetically modified to remove the PPARα. Several mechanisms have been proposed to explain how, in rodents,

peroxisome proliferation can lead to the formation of hepatocellular tumors. The general consensus of scientific opinion is that PPARα-induced mitogenesis and cell proliferation are probably the major mechanisms responsible for peroxisome proliferator-induced hepatocarcinogenesis in rodents. 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. Rats and mice are uniquely responsive to the morphological, biochemical, and chronic carcinogenic effects of peroxisome proliferators, while guinea pigs, dogs, nonhuman primates, and humans are essentially nonresponsive or refractory; Syrian hamsters exhibit intermediate responsiveness. These differences are explained, in part, by marked interspecies variations in the expression of PPARα, with levels of expression in humans being only 1-10% of the levels found in rat and mouse liver (Regulatory Toxicology and Pharmacology, 20, 327-357, 1999).

The Registrant submitted the following studies in support the role of CGA 184927 as a peroxisome proliferator and its role in induction of peroxisomal and microsomal enzymes, and 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, CGA 184927 increased the liver weights in both sexes of rat at 1000 ppm and in males at 120 ppm. Recovery in liver weights was noted after a treatment-free period. The 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 increase in all 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 slight but statistically significant 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) Bieri, F. (1991). Effects on selected plasma hormone concentrations and biochemical parameters in the liver upon subchronic administration to male adult rats. MRID NUMBER: Not assigned

CGA 184927 has been identified as a rodent-specific peroxisomal proliferating agent. This study investigated CGA 184927-induced alteration of the steroid hormone metabolism in male Tif:RAIf (SPF) rats. Dietary administration of the test compound to 10 rats at dose levels of 750 ppm (53.5 mg/kg/day) for 14 days produced increase in liver weights (absolute: 134% and relative: 139% of controls). [Controls received normal diet during the study period]. No changes were seen in the organ weights of endocrine or sex hormone producing glands. A significant increase (807% of control) in liver peroxisomal fatty acid beta-oxidation activity and a moderate to strong increase in liver microsomal cytochrome P450 isoenzymes CYP4A1/A3 (242% of control) and CYP4A2 (618% of control). Furthermore, a decrease in CYP2A, CYP3A, and male-specific CYP2C11 was observed. Treatment-related decrease was noted in total liver microsomal testosterone oxidation rate to 34% of control; whereas, aromatase (CYP19A1) activity was increased to 169% of control with a resulting increase in plasma total estradiol (170% of control), and plasma 5 alpha-dihydrotestosterone (198% of control) concentrations.

These findings confirmed that CGA 184927 is a potent peroxisome proliferator in the rat liver. This rodent-specific peroxisomal proliferating activity manifests itself by altering cytochrome P450-dependent monooxygenases in the liver which are involved in steroid hormone homeostasis in a manner analoguous to that described for other peroxisomal proliferating agents.

D) 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. During the treatment period, food consumption, body weight and clinical signs were recorded. On day 4 of treatment and prior to sacrifice, blood samples were collected. At necropsy, liver, endocrine glands and accessory sex organs were frozen and stored at -80°C. The biochemical as well as hormonal parameters were determined in the appropriate subcellular liver fractions or in blood plasma according to published procedures. The parameters examined included liver protein content, liver microsomal regio-and stereoselective testosterone hydroxylation, liver microsomal cytochrome P450 CYP19A1 (aromatase), liver peroxisomal fatty acid β-oxidation, plasma dihydrotestosterone, free and total plasma testosterone, plasma total estradiol, and plasma follicle stimulating hormone, prolactin and luteinizing hormone.

Treatment with CGA 184927 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 peroxisome proliferating agent and alters monooxygenase activity in subfamilies of cytochrome P450 which are known to

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be involved in the synthesis or catabolism of steroid hormones.

E) 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, a peroxisome proliferator, is rapidly hydrolysed 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.

F) 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 benzafibric 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.

G) Roberts R. (1999). CGA-193469 (free acid derivative of CGA-184927) peroxisome proliferators: species difference in the regulation of gene expression (MRID not assigned).

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 constrast, CGA 193469 was unable to activate the human ACO promoter-reporter construct.