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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: RfD/Peer Review Report of Alpha-Metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl).

CASRN: 87392-12-9
EPA Chem. Code: 108800
Caswell No.:

FROM: George Z. Ghali, Ph.D. GGhali
Manager, RfD/QA Peer Review Committee
Health Effects Division (7509C)

THRU: William Burnam
Co-Chairman, RfD/QA Peer Review Committee
Health Effects Division (7509C)

TO: Joanne Miller, PM 23
Fungicide-Herbicide Branch
Registration Division (7505C)

The Health Effects Division-RfD/Peer Review Committee met on April 10, 1997 to discuss and evaluate recently submitted toxicology data in support of Alpha-Metolachlor (S-enantiomer, CGA 77102) registration.

Material available for review consisted of data evaluation records (DERs) for developmental toxicity studies in rats and rabbits **(83-3a and -3b)**, subchronic toxicity studies in rats and dogs **(82-1a and -1b)**, and a battery of mutagenicity studies **(84-2)**.

A. Background:

Alpha-metolachlor (CGA 77102) is the S-enantiomer of the racemic compound Metolachlor (CGA 24705). Technical grade Metolachlor, which is currently registered with the Agency, consists of 50% of each of the R-enantiomer (CGA 77101) and the S-enantiomer (CGA 77102).

The registrant has proposed the use of the toxicology data base, submitted in support of the registration of technical grade Metolachlor, to support the registration of Alpha-metolachlor. The registrant's request was based on the fact that the S-enantiomer, Alpha-metolachlor, has already been subject to extensive toxicological testing during the course of the development of Metolachlor.

Therefore, the specific investigation of Alpha-metolachlor submitted to the Agency was confined to selected endpoints in the area of acute and subchronic toxicity, mutagenicity and reproductive toxicity for the purpose of identifying possible qualitative or quantitative differences between the toxicological properties of Alpha-Metolachlor and Metolachlor.

The Committee was asked to determine whether the limited toxicological investigation submitted on behalf of Alpha-metolachlor is adequate to demonstrate that both alpha-metolachlor and Metolachlor itself have identical toxicological properties, and if so, the applicability of the data base established for Metolachlor in the safety evaluation of the Alpha-metolachlor, and whether or not a separate Reference Dose (RfD) for this chemical should be established in this case.

B. Chronic and Subchronic Toxicity:

I. Alpha-Metolachlor:

There were no chronic toxicity data in rats (83-1a) or dogs (83-1b) available for review by the Committee. Two subchronic studies in rats and dogs (82-1a, 1995, MRID No. 43928923 and 82-1b, 1995, MRID No. 43928922) were available for review by the Committee. The Committee considered both subchronic toxicity studies to be acceptable and the data evaluation records (HED Doc. No. 000000) to be adequate.

The Committee considered the subchronic toxicity study in rats (82-1a, 1995, MRID No. 43928923) to be acceptable and the data evaluation record (HED Doc. No. 000000) to be adequate.

In this study, actually conducted in 1983, alpha-metolachlor (89.6%) was administered to Sprague-Dawley rats at dietary levels of 30, 300, 3000 or 10,000 ppm (1.5, 15, 150 or 500 mg/kg/day). The NOEL/LOEL were established at 15 and 150 mg/kg/day, respectively, based on lower body weights and body weight gains, reduced food consumption and reduced food efficiency in both sexes and increased kidney weights in males.

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The Committee considered the subchronic toxicity study in dogs (82-1b, 1995, MRID No. 43928922) to be acceptable and the data evaluation record (HED Doc. No. 000000) to be adequate.

In this study, alpha-metolachlor (95.4%) was administered to beagle dogs at dietary levels of 300, 500, 1000 or 2000 ppm (9, 15.1, 31.1 and 62 mg/kg/day in males and 10, 17.2, 31.5 or 74 mg/kg/day). The NOEL was considered to be 2000 ppm (62 and 74 mg/kg/day in males and females, respectively), the highest dose level tested.

II. Metolachlor:

In a subchronic study conducted with Metolachlor, groups of Sprague-Dawley rats were administered Metolachlor in diets at 100, 300 or 1000 ppm (5, 50 or 50 mg/kg/day) for three months. Since no toxic manifestations were evident in any group at week ten, it was decided to increase the dose from 100 ppm to 2000 ppm for the remaining three weeks of the study. Ten rats per sex from the high- dose group also received increased levels of 2000 ppm for the remaining three weeks of the study and then sacrificed after a recovery period of 4 weeks. No significant systemic effects were noted at any dose tested. Therefore, the NOEL was established at 1000 ppm (50 mg/kg/day), the highest dose level tested.

C. Reproductive and Developmental Toxicity:

I. Alpha-Metolachlor:

The Committee considered the developmental toxicity study in rats (83-3a, 1995, MRID 43928925) to be acceptable and the data evaluation record (HED Doc No. 000000) to be adequate.

In this prenatal developmental toxicity study in Tif: RAI f (SPF) rats (24/group), alpha-metolachlor (95.6%) was administered by gavage in aqueous 0.5% carboxymethylcellulose at a dose volume of 10 ml/kg on gestation days 6-15; dams were c-sectioned on gestation day 21. Dose levels were 5, 50, 500, or 1000 mg/kg/day. The maternal NOEL was 50 mg/kg/day, and the maternal LOEL was 500 mg/kg/day, based on increased clinical signs (pushing head through bedding for approximately one hour after dosing), decreased body weights and weight gain, and reduced food consumption and food efficiency. The developmental NOEL was $\geq$1000 mg/kg/day (no treatment-related developmental toxicity was observed).

The Committee agreed with the reviewer that an apparent decrease in litter size at 1000 mg/kg/day is equivocal and should not be attributed to treatment.

The Committee considered that the developmental toxicity study in rabbits (83-3b, 1995, MRID 43928924) to be acceptable and the data evaluation record (HED Doc. No. 000000) to be adequate.

In this prenatal developmental toxicity study, actually conducted in 1983 in New Zealand White rabbits (19/group), 89.6% alpha-metolachlor (93.7% S isomer) was administered by gavage at doses of 20, 100, or 500 mg/kg/day on D 7-19. The test substance was delivered in 3% corn starch in 0.5% Tween 80 at a dose volume of 10 ml/kg. The maternal NOEL was 20 mg/kg/day, and the maternal LOEL was 100 mg/kg/day, based on clinical signs of toxicity (soft stool). At 500 mg/kg/day, maternal body weight, weight gain, food consumption, and food efficiency were reduced during treatment, with a rebound increase following cessation of dosing. The developmental NOEL was considered to be ≥500 mg/kg/day. There was no evidence of developmental toxicity observed in this study.

The Committee considered the statistically significant increase in the fetal and litter incidence of full 13th ribs, and agreed with the reviewer, that this finding was not biologically relevant, since a dose-related response was not apparent and because the absolute number of affected litters was equivalent between the high-dose and control groups.

II. Metolachlor:

In a two-generation reproduction study in Sprague-Dawley rats, 94.5% metolachlor was administered at dietary levels of 30, 300, or 1000 ppm (2.3, 23.6, or 76.2 mg/kg/day for males; 2.5, 25.9, or 85.1 mg/kg/day for females). The parental systemic NOEL was 1000 ppm (76.2/85.1 mg/kg/day for M/F), the highest dose tested. The reproductive NOEL was 300 ppm (25.9 mg/kg/day for dams) and the reproductive LOEL was 1000 ppm (85.1 mg/kg/day for dams), based on decreased offspring postnatal body weights on days 14 and 21 for F1a litters and days 4, 7, 14, and 21 for F1b litters (Page, 1981; MRID 00080897).

In a prenatal developmental toxicity study in Sprague-Dawley rats (25/group), metolachlor (96.4%) was administered by gavage in aqueous 0.5% carboxymethylcellulose at a dose volume of 10 ml/kg on gestation days 6-15. Dose levels were 30, 100, 300, or 1000 mg/kg/day. The maternal NOEL was 100 mg/kg/day, and the maternal LOEL was 300 mg/kg/day, based on clinical observations of salivation. At 1000 mg/kg/day, the following were observed: mortality (4/25), more extensive clinical signs (salivation, urine stained abdominal fur, excess lacrimation, and clonic or clonic/tonic convulsions), and reductions in body weight gain and food consumption. The developmental NOEL was 300 mg/kg/day, and the developmental LOEL was 1000 mg/kg/day, based on decreased implantations/dam, reduced litter size, increased postimplantation loss (resorptions/dam), and decreased mean fetal body weight (Lochry, 1985; MRID 00151941).

In a previously-conducted prenatal developmental toxicity study in Sprague-Dawley rats (25/group), metolachlor (unspecified purity) was administered by gavage in 2% carboxymethylcellulose at dose levels of 60, 180, or 360 mg/kg/day on gestation days 6-15. No evidence of

maternal or developmental toxicity was observed at any dose level. (Fritz, 1976; MRID 00015396)

In a prenatal developmental toxicity study in New Zealand White rabbits (16/group), 95.4% metolachlor was administered by gavage at doses of 36, 120, or 360 mg/kg/day on D 7-18. The test substance was delivered in 0.75% aqueous hydroxymethylcellulose at a dose volume of 10 ml/kg. The maternal NOEL was 120 mg/kg/day. The maternal LOEL, 360 mg/kg/day, was based on increased clinical observations (blood in pan and anorexia) and reduced body weight gain. No evidence of developmental toxicity was noted. Therefore, the developmental toxicity NOEL was considered to be ≥360 mg/kg/day (Lightkep, 1980; MRID 00041283).

III. Developmental Neurotoxicity:

Based upon a review of the currently available data base for alpha-metolachlor, a developmental neurotoxicity study in rats is not recommended at this time.

IV. Conclusions (Reproductive and Developmental Toxicity):

In the prenatal developmental toxicity studies, administration of Alpha-metolachlor at limit dose levels (1000 mg/kg/day) did not result in any evidence of developmental toxicity. Studies conducted with Metolachlor were not sufficiently equivalent in dose spacing to allow a comparison of the study findings. Furthermore, the results of the two-generation study in rats with metolachlor can not be extrapolated to alpha metolachlor due to the lack of available supporting or bridging toxicity information.

D. FOPA Considerations:

The data package for Metolachlor included acceptable prenatal developmental toxicity studies in rats and rabbits with alpha-metolachlor. In addition, an acceptable two-generation reproduction study in rats and acceptable prenatal developmental toxicity studies in rats and rabbits were submitted for metolachlor. The completeness of this data package, specifically in regard to the evaluation of Alpha-metolachlor for reproductive toxicity, is dependant upon the adequacy of standard "bridging" data; these data were not addressed by the Committee.

The data provided no indication of increased sensitivity of rats or rabbits to *in utero* exposure to alpha-metolachlor. No evidence of developmental toxicity was observed in either species, at dose levels which were demonstrated to be maternally toxic. In the prenatal developmental toxicity study in rats, the developmental NOEL was ≥1000 mg/kg/day, the limit dose, although maternal toxicity was observed at 500 mg/kg/day (maternal NOEL = 50 mg/kg/day). In the prenatal developmental toxicity study in rabbits, the developmental NOEL was ≥500 mg/kg/day, while the maternal NOEL was 20 mg/kg/day, based on

clinical signs of toxicity (soft stool) at the LOEL of 100 mg/kg/day.

Additionally, the data provided no indication of increased sensitivity of rats or rabbits to *in utero* exposure to metolachlor. In the prenatal developmental toxicity study in rats, the maternal NOEL (100 mg/kg/day) was less than the developmental NOEL (300 mg/kg/day). Developmental toxicity noted at the highest dose tested, 1000 mg/kg/day, occurred in the presence of severe maternal toxicity. In the prenatal developmental toxicity study in rabbits, no developmental toxicity was observed, although maternal toxicity was noted at the LOEL of 360 mg/kg/day (increased clinical signs and reduced weight gain).

However, in the two-generation reproduction study in rats, a possible sensitivity of the offspring to *in utero* and/or postnatal exposure to metolachlor is suggested by the data. Although no parental systemic toxicity was identified, a reproductive NOEL (300 ppm; 25.9 mg/kg/day for dams) was based on decreased offspring postnatal body weights (days 14 and 21 for F1a litters and days 4, 7, 14, and 21 for F1b litters) at the reproductive LOEL of 1000 ppm (85.1 mg/kg/day for dams).

E. Mutagenicity:

Three acceptable mutagenicity studies (84-2) with alpha-metolachlor were available for review by the Committee. The following is a summary of these studies and the Committee's conclusions for each study:

I. Gene Mutation:

Salmonella typhimurium/Escherichia coli reverse gene mutation assay (MRID No. 43928927): Independently performed tests were negative up to cytotoxic doses (≥1250 ug/plate+/- S9 in strains S. Typhimurium TA1535, TA1537, TA100 and TA102 or 5000 ug/plate +/- S9 in strains S. typhimurium TA98 and E. coli WP2 uvrA).

II. Chromosome Aberrations:

In vivo bone marrow micronucleus assay (MRID No. 43928928): The test was negative in Tif:MAGf(SPF) mice up to the highest dose tested (2000 mg/kg/day) when administered once by oral gavage. Overt toxicity (ataxia, tremors and/or hunched posture) but no bone marrow cytotoxicity was seen in the high-dose group.

III. Other mutagenic mechanism:

In vivo/in vitro replicative DNA synthesis (RDS)/unscheduled DNA synthesis (UDS) in rat hepatocytes (MRID No. 43928928): The test was negative for UDS in Tif: RAIf (SPf) rats at a dose (1500 mg/kg/day) that caused a marked increase in RDS (cellular proliferation). Death and/or cytotoxicity in survivors occurred at levels ≥ 3200 mg/kg.

IV. Conclusions (Mutagenicity):

Results from the three studies indicated that alpha-metolachlor was neither mutagenic to microbial cells nor clastogenic in whole animals. Similarly, there was no evidence of DNA damage/repair in the hepatocytes recovered from treated rats. In contrast, treatment with alpha-metolachlor induced marked increase in cell proliferation indicating that the test substance reached the target organ and induced a hepatotoxic but not genotoxic effect. Overall, the findings with alpha-metolachlor are in good agreement with the genetic toxicology profile for metolachlor. It was noted that an in vitro mammalian cell gene mutation assay was not included in the data package. However, an acceptable and negative mouse lymphoma assay with metolachlor was previously submitted to the Agency. Based on the chemical equivalency and similarity of the genetic toxicology profiles for alpha-metolachlor and metolachlor, we concluded that the requirement to conduct a mammalian cell gene mutation assay can be waived.

The Committee further concluded that the submitted test battery satisfies the new mutagenicity initial testing Guidelines. No other genetic toxicology data requirements have been identified at this time.

F. Reference Dose (RfD):

The Committee deferred the decision on whether or not a separate Reference Dose (RfD) for Alpha-metolachlor should be established until a final decision is made regarding the adequacy of the toxicology data base and whether it is appropriate to use toxicology data generated with Metolachlor to support the registration of Alpha-metolachlor.

G. Committee's Conclusions and Recommendations:

The Committee compared data available on Alpha metolachlor with those submitted in support of Metolachlor registration and concluded that without metabolism studies and side-by-side subchronic studies conducted in the same strain of rat using comparable dose levels of the subject test substances, the identification of any possible qualitative or quantitative differences between the toxicological properties of CGA 77102 and metolachlor would not be possible.

Therefore, the Committee could not determine whether the use of the toxicology data base, submitted in support of the registration of technical grade metolachlor, to support the registration of Alpha-metolachlor would be appropriate at this time.

G. Individuals in Attendance:

Peer Review Committee members and associates present were William Burnam (Chief, SAB; Chairman, RfD/Peer Review Committee), George Ghali (Manager, RfD/Peer Review Committee), Karl Baetcke (Chief, TB I), Mike Ioannou (Acting Chief, TB II), Kit Farwell, Guruva Reddy, and Henry Spencer. In attendance also was Kathryn Boyle of HED as an observer.

Scientific reviewers (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report):

Stephen Dapson

Jess Rowland

Respective Branch Chief (Committee member; signature indicates concurrence with the peer review unless otherwise stated)

Mike Ioannou

CC: Stephanie Irene
Debra Edwards
Mike Ioannou
Jess Rowland
Stephen Dapson
Amal Mahfouz (OW)
RfD File
Caswell File

H. Material Reviewed:

1. Khalil, S. (1995). CGA-77102 Rat Oral Teratology. MRID No. 43928925. HED Doc. No. 000000. Classification: Acceptable.

2. Gilles, P. A. and Giknis, M. L. A. (1995). A Teratology Study of CGA-77102 Technical in New Zealand White Rabbits. MRID No. 43928924. HED Doc. No. 00000. Classification: Acceptable.

3. Chang, J.C.F. (1995). CGA-77102 Technical 13-Week Oral Toxicity in Rats. MRID No. 43928923. HED Doc. No. 000000. Classification: Acceptable.

4. Chang, J. C. F. (1995). CGA-77102 Technical Final Report 90-Day Oral Toxicity in Dogs. MRID No. 43928922. HED Doc. No. 000000. Classification: Acceptable.

5. Hertner, Th. (1995). CGA-77102 Technical In Vivo/In Vitro Unscheduled DNA Synthesis in Rat Hepatocytes. MRID No. 43928928. HED Doc. No.000000. Classification: Acceptable.

6. Hertner, Th. (1995). CGA-77102 Technical: In vivo/in vitro unscheduled DNA synthesis in rat hepatocytes. MRID No. 43928928, HED Doc. No. 000000. Classification: Acceptable.

7. Hertner, Th. (1995). CGA-77102 Technical: Micronucleus test, mouse (OECD conform). MRID No. 43928926, HED Doc. No. 000000. Classification: Acceptable.

8. Hertner, Th. (1995). CGA-77102 Technical: Salmonella and Escherichia/mammalian-microsome mutagenicity test. MRID No. 43928927, HED Doc. No. 000000. Classification: Acceptable.