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

005504

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metolachlor Registration Standard, Transmittal of the Toxicology Chapter

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FROM: Robert P. Zendzian PhD
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6.2.86

Attached is the Toxicology Chapter of the Metolachlor Registration Standard. This document has been constructed by taking those parts of the 1980 Metolachlor standard that refer to studies that were accepted and are still valid, deleting those portions that refer to data gaps that have been filled and adding information on new data that has been received since 1980. The DERs included are only for the new studies received since 1980.

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Metolachlor Toxicology Chapter

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A. Toxicology Profile

81 Series Acute toxicity and Irritation Studies

81-1 Acute Oral Toxicity

The minimum testing needed on acute oral toxicity is one test on the laboratory rat for the technical material. For Technical Metolachlor, the acute oral LD_{50} in the laboratory rat is 2,780 mg/kg with 95% confidence limits of 2,180-3,545 mg/kg (MRID 15523). Technical Metolachlor in corn oil has been shown to be emetic in beagle dogs to an extent that precludes the establishment of an oral LD_{50} in dogs (MRID 15525). The study did, however, establish the 'emetic dose 50' to be 19.0 (+/- 9.7) mg/kg. The Technical material is in Toxicity Category III with regard to acute oral toxicity.

81-2 Acute Dermal Toxicity

The minimum testing needed on acute dermal toxicity is one test, preferably on the albino rabbit, for the technical material. The dermal LD_{50} of technical Metolachlor on the New Zealand rabbit is greater than 10,000 mg/kg when tested by the unabraded dermal route (MRID 15526). This data is sufficient to meet the requirement for acute dermal toxicity data on intact skin. The unabraded dermal test results place technical Metolachlor in Category III with respect to acute dermal toxicity.

81-3 Acute Inhalation Toxicity

The minimum data needed on acute inhalation toxicity is one acute inhalation study, using one mammalian species, preferably the albino rat. An acute inhalation toxicity study of technical Metholachlor showed no deaths in albino rats at the maximum achievable level of exposure (1.752 mg/l with four hours of exposure) (MRID 15535). This study is adequate to establish a Toxicity Catagory IV for inhalation exposure for Technical Metolachlor.

81-4 Primary Eye Irritation

The minimum testing needed to evaluate eye irritation potential is one test for the technical material, conducted on the albino rabbit. A study of eye irritation for the Technical was conducted on the New Zealand rabbit (MRID 15526). In that study 0.1 ml of Technical Metolachlor was used. The test was evaluated using the system of Draize (1959) and produced the following eye irritation and indices at 24 hours and 7 days:

Cornea:	0
Iris:	0
Conjunctivae:	0

This study establishes that Technical Metolachlor is non-irritating to the rabbit eye (Category IV).

81-5 Primary Dermal Irritation

The minimum testing needed to determine the potential for primary dermal irritation is one test conducted on a mammal, preferably the albino rabbit, for the technical material. Technical Metolachlor was evaluated for dermal irritation on the New Zealand rabbit (MRID 15530). The test was evaluated using the system of Draize and resulted in a primary irritation index of 0.1. This information is sufficient, and it establishes that Technical Metolachlor is non-irritating to rabbit skin (Category IV).

81-6 Dermal Sensitization

The minimum data needed to assess dermal sensitization can be provided by an intradermal test on one mammalian species, preferably the male albino guinea pig. In a study using the intradermal injection method, technical Metolachlor dissolved in the vehicle (propylene glycol) and the vehicle alone (negative control) were intradermally injected into the skin of Pilbright guinea pigs (MRID 15631). Positive reaction was demonstrated in animals injected with Technical Metolachlor dissolved in the vehicle; there was no reaction in animals injected with the vehicle alone. Based on this study it is established that Technical Metolachlor is a skin sensitizer in guinea pigs.

81-7 Acute Delayed Neurotoxicity

This type of data is needed only if the active ingredient is an organophosphate and it or any of its metabolites, degradation products, or impurities cause esterase depression or are structurally related to a substance that induces the specific neuropathy, organophosphate type delayed neurotoxicity. Metolachlor is a chloroacetanilide herbicide. This test is not required for Technical Metolachlor.

82 Series Subchronic Testing

82-1 Subchronic Oral Dosing

Testing should be performed in at least 2 mammalian species. One species should be a generally recognized strain of laboratory rat while the second species should be a non-rodent.

Three-month feeding studies were performed with Sprague Dawley rats (MRID 15674) and with beagle dogs (MRID 17690). The Agency determined that the histopathology evaluations for both the rat and the dog study were not performed by a pathologist. Subsequently, the histopathology from the 90-day dog study was re-read by a qualified pathologist, and this re-evaluation of histopathology was submitted to the Agency, allowing this study to fulfill the Guideline's requirement for non-rodent subchronic oral dosing.

In the three-month feeding study in dogs (MRID 17690) four male and four female beagle dogs were assigned to four treatment groups and dosed with 0, 50, 150 and 500 ppm of metolachlor in the diet. After eight weeks on test the 50 ppm group waa switched to 1000 ppm metolachlor. Six additional animals (3M and 3F) were carried in the control (four) and high dose (two) groups. Parimeters observed were generally those required in the guidelines for subchronic studies. Except for a decrease in food consumption and associated small weight loss at the high dose, no compound-related effects were observed.

Because an acceptable two-year rat chronic feeding study has been received the Agency has decided to waive the need for a re-evaluation of the histopathology of the 90-day feeding study on rats (MRID 15674).

A six-month (180 day) dog study (MRID 16632) was performed in support of certain tolerance petitions in lieu of adequate chronic data. Equal numbers of males and females were dosed with Metolachlor at 0 (8/8), 100 (6/6), 300 (6/6) and 1000 (8/8) ppm in the diet. Possible compound-related effects consisted of a decreased gain in body weight in males and females and a failure of the serum alkaline phosphatase to decrease with increasing age in both sexes. It was concluded that the 'no observed effect level' (NOEL) in the study was 100 ppm.

Upon reevaluation of this study it was noted that the statistically significant decreases in activated partial thromboplastin time (APTT) observed in males and females may not have been due to error in methodology. The decreases appear to be dose and time related with indications of a trend. This is particularly apparent when the data are converted to percent of concurrent control. This evaluation does not change the previously established NOEL of 100 ppm in this study. However additional evaluation of the effects of Metolachlor on the clotting system of the dog are required to determine if this is a compound-related effect (Zendzian 1986).

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Memo, R.P. Zendzian, Metolachlor, Effect on Coagulation in a Six-Month Dog Study, May 1986.

82-2 Subchronic Dermal (21-day)

This study is not needed because the existing acceptable end-uses should not result in repeated human skin contact for extended periods.

82-3 Subchronic Dermal (90-day)

This study is not needed because the existing acceptable end-uses should not result in repeated human skin contact for extended periods.

82-4 Subchronic Inhalation

The existing acceptable end uses should not result in repeated inhalation exposure. This study is not required.

82-5 Subchronic Neurotoxicity

An acute neurotoxicity study is not required on Metolachlor and therefore, this study is not required.

83 Series Chronic and Long Term Studies

83-1 Chronic Toxicity

Chronic testing should be available on at least one mammalian species. The species should normally be a generally recognized strain of the laboratory rat.

One two-year feeding study on the rat was performed (MRID 15634), but the Agency found the study to be invalid because of several deficiencies in protocol, including the fact that dose levels were not verified by an analysis of the diet. The study does offer supplementary information on Metolachlor's potential oncogenicity (see below).

A second two-year feeding study in has been submitted that is acceptable (MRID 63398). Doses were 30, 300 and 3000 pmm. Mean body weights of the high-dose females were consistantly lower then controls from the second week until termination. The difference was significant ($p < 0.01$) for 26 of the 59 weights taken. Testicular atropy was observed in the high and mid dose males with a NOEL of 30 ppm.

83-2 Oncogenicity

For the adequate assessment of oncogenicity, studies are needed in two mammalian species: normally, the mouse and the laboratory rat.

A mouse study was conducted with Charles River CD-1 albino mice (50 of each sex) at levels of 0, 30, 1,000, and 3,000 ppm fed in the diet (MRID 84003) The duration of the study was 18 months for males and 20 months for females. It was conducted by Industrial Bio-Test Laboratories (IBT) and validated by Ciba-Geigy Corporation Because EPA suspected that some of the toxicology studies performed by IBT were deficient to the point of not being valid for the support of pesticide registrations, Ciba-Geigy initiated a new mouse oncogenicity study. However, the Agency's subsequent in-depth evaluation of the IBT study found that, despite certain deficiencies in good laboratory practices and animal husbandry techniques, the raw data supported the reported negative results. The IBT study therefore satisfies the requirement for mouse oncogenicity testing, and the Agency concludes from it that Metolachlor did not show an oncogenic effect at the given dietary dosages.

A second oncogenic study in mice was submitted in 1980 (MRID 39194). Mice were dosed for 104 weeks with Metolachlor at 300, 1000 and 3000 ppm in the diet. "The study is negative, as no increase in tumors was noted at the HDT, 3000 ppm. A decrease in body weight gain of high dose males and females was noted, indicating that 3000 ppm was a Maximally Tolerated Dose (MTD). No other significant chronic effects were noted in this study.'

Though the two-year chronic feeding study on rats discussed in the 'Chronic Effects' section above) (MRID 15418) was not valid for the fulfillment of the chronic feeding data requirement, it did offer supplementary information that Metolachlor is oncogenic. "An increase in primary liver tumors was found in high dose female rats. In this study hyperplastic nodules were included as an oncogenic response based on recommendations of the National Cancer Institute that hyperplastic nodules be classified as neoplastic nodules."

The second two-year feeding study discussed above (MRID 63398) satisfies the requirement for an oncogenic study in the rat. "A significantally increased incidence of proliferative hepatic lesions was found in high dose females at terminal sacrifice."

These observations are discussed in detail in the section on Toxicological Issues:

83-3 <u>Teratogenicity</u>

The minimum data needed to evaluate the potential fetotoxic or teratogenic effects of a pesticide are tests in two mammalian species.

A study of the teratogenic effects of Technical Metolachlor was conducted on rats (MRID 15396). The study found that oral doses of either 60, 180, or 360 mg/kg/day during 6 to 15 days of gestation did not affect the offspring of female Sprague-Dawley rats. No fetotoxic effects of the compound were observed. The only possible effect on the rats was a decrease in food consumption at the highest dose during the first one-third of the experiment which may indicate that this was the beginning of toxic maternal doses. This study is sufficient for the assessment of teratology in one species of mammal, and does not show any evidence of a teratogenic hazard for Metolachlor.

A teratogenic study study was submitted on Metolachlor in New Zealand White Rabbits (MRID 41283). Doses used were 36, 120 and 360 mg/kg/day orally. Maternal toxicity was observed at the high dose. No evidence of compound induced fetotoxicity or teratogenicity was observed. This study satisfies the the requirement for a teratogenicity study in a nonrodent species.

83-4 Reproduction

The minimum data needed for measuring reproductive effects can be provided by one rat study lasting two generations. A two-generation reproduction study of metolachlor in Charles River rats shows no direct effect on reproduction at dietary doses up to 1000 ppm (MIRD 80897). Metolachlor technical was fed in the diet at doses of 0, 30, 300 or 1000 ppm for a standard two-generation, one litter per generation, reproduction study. The study is classified Core Guideline, " The NOEL for reproductive effects is 300 ppm based on reduced pup weights and reduced parental food consumption at 1000 ppm. Other effects that may be related to treatment were increased liver to body weight and thyroid to body weight ratios in the 1000 ppm F_1 parents." The effect on pup body weights at 1000 ppm can be considered secondary to the reduced food consumption of the dams. This study satisfies the requirement for a reproduction study on technical Metolachlor.

84 Series Mutagenicity

84-2 Mutagenicity

In order to assess the potential of Metolachlor to to affect the qualitative and quantitive integrity of human genetic material, a battery of tests is normally required to address three categories of possible genetic effects: 1) gene mutation, 2) structural chromosomal aberrations and 3) other mutagenic mechanisms as deemed appropriate.

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The potential of Metolachlor to cause genetic changes has been tested for in a bacterial system utilizing activation by mammalian microsomes (MRID 15397). The bacterial (Salmonella) system was tested for base substitutions and point mutations at various ranges (10, 100, 1,000 and 10,000 ug/plate). No increase in background mutation rates was observed. This study partially satisfies the requirement for a gene mutation test. Additional testing is required in an in vitro mammalian cell system.

The potential of Metolachlor to cause genetic changes has been tested for in a dominate lethal test in the mouse (MRID 15630). No effects wore noted in the mouse study, on fertility rates, or on zygote or embryo survivals, after single oral doses of 100 and 300 mg/kg. Also, no malformations of resulting embryos were reported. This study is applicable to the requirements for gene mutation and structural chromosomal aberration. However, additional testing is required because of the relative insensitivity of the test.

The ability of Metolachlor to cause genetic effects was evaluated in the micronucleous test in chinese hamsters (MRID RPZ0001). The study was classi ied as 'inconclusive' "because data were not submitted to demonstrate that the test article reached the target tissue, bone marrow."

A primary DNA damage/repair assey was conducted with Metolachlor on human fibroblasts (MRID RPZ0002). The study was classified as 'Unacceptable'.

"The study is deficient because range-finding data were not submitted to support the selection of test article concentrations, nor was any evidence of cytotoxicity presented in the main study. Current guidelines for in vitro mutagenicity studies require that chemicals be tested to the limits of cytotoxicity or solubility. Also. the effect of metabolic activation was not assesed."

A primary DNA damage/repair assey was conducted with Metolachlor on rat hepatocytes (MRID RPZ0003). The study was classified as 'Unacceptable'.

"The study is deficient because range-finding data were not submitted to support the selection of test article concentrations, nor was any evidence of cytotoxicity presented in the main study. Current guidelines for in vitro mutagenicity studies require that chemicals be tested to the limits of cytotoxicity or solubility."

In summary, additional testing is required for, 1) in vitro gene mutation in a mammalian cell system, 2) chromosomal aberration and 3) direct DNA damage.

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85 Series Special Studies

85-1 Metabolism

Three reports of metabolism studies of Metolachlor in the rat have been received (MRID 15654, 15655 and 39293). All three reports are unacceptable as they are lacking in individual animal data. Report MRID 15654 may be able to satisfy that portion of the metabolism Guidelines on a single low oral dose to rats otherwise it can at best be supplimentary. Reports MRID 15655 and 39193 are of the same study and may be classifiable a supplementary being a preliminary identification of metabolites of Metolachlor in urine and feces.

At best these studies will not satisfy the requirements for metabolism studies of Metolachlor. Additional testing is required.

B. Data Gaps

Metolachlor is registered for use on food crops and has food tolerances. The following Guideline toxicology studies can be required for this registration.

81-1 Acute Oral Toxicity
81-2 Acute Dermal Toxicity
81-3 Acute Inhalation Toxicity
81-4 Primary Eye Irritation
81-5 Primary Dermal Irritation
81-6 Dermal Sensitization
81-7 Acute Delayed Neurotoxicity

82-1 Subchronic Oral Dosing
in two species
82-2 Subchronic Dermal (21-day)
82-3 Subchronic Dermal (90-day)
82-4 Subchronic Inhalation

83-1 Chronic Toxicity
83-2 Oncogenicity
in two species
83-3 Teratogenicity
in two species

83-4 Reproduction

84-2 Mutagenicity

85-1 Metabolism

Based on this assesment of the toxicology data base for Metolachlor the following Guideline Toxicology studies have been identified as data gaps and are required.

84-2 Mutagenicity

This data requirement is only partially satisfied and additional testing is required for; 1) in vitro gene mutation in a mammalian cell system, 2) chromosomal aberration and 3) direct DNA damage.

85-1 Metabolism

Based on this assesment of the toxicology data base for metolachlor the following additional nonguideline study is required.

85 Special studies

The Registrant is required to design and perform studies to investigate the effect on Metolachlor in the clotting system in the dog.

The six month dog feeding study showed statistically significant decreases in activated partial thromboplastin time (APTT) in a dose and time related fashion in both sexes. Considering the rarity of this observation, the potential for harm of increased intravascular coagulation and the questions raised as to its 'reality', the Agency is requiring that the Registrant perform studies in the dog to investigate the effect(s) of Metolachlor on coagulation system.

C. Tolerances and Tolerance Reassesment

Tolerances for Metoalchlor have been approved for the RACs listed.

Published Tolerances

Crop	Tolerance (ppm)	Food Factor	mg/day(1.5kg)
Corn,grain	0.100	1.00	0.00150
Soybeans (oil)	0.100	0.92	0.00138
meat, inc poultry	0.020	13.85	0.00415
Milk & Dairy Products	0.020	28.62	0.00858
Eggs	0.020	2.77	0.00083
Peanuts	0.100	0.36	0.00054
Sorgum	0.300	0.03	0.00014
Barley	0.100	0.03	0.00005
Buckwheat	0.100	0.03	0.00005
Millet	0.100	0.03	0.00005
Milo	0.100	0.03	0.00005
Oats	0.100	0.36	0.00054
Rice	0.100	0.55	0.00083

Rye	0.100	0.03	0.00005
Wheat	0.100	10.36	0.01554
Corn, sweet	0.100	1.43	0.00215
Cottonseed (oil)	0.100	0.15	0.00022
Potatoes	0.200	5.43	0.01628
Safflower	0.100	0.03	0.00005
Seed & Pod Veg	0.300	3.66	0.01646
Chili Peppers	0.500	0.03	0.00023

Toxicology Branch Approved but Unpublished Tolerances

Sunflower	0.300	0.03	0.00014
Corn, Pop	0.100	0.03	0.00012
Liver	0.050	0.03	0.00002
Kidney	0.200	0.03	0.00009
Peanuts	0.400	0.36	0.00215
Stone Fruits	0.100	1.25	0.00187

The initial ADI for Metolachlor was based on a six-month dog feeding study (MRID 16632). Compound-related effects consisted of a decreased gain in body weight in males and females and a failure of the serum alkaline phosphatase to decrease with increasing age in both sexes. The 'no observed effect level' (NOEL) in the study was 100 ppm (2.5 mg/kg). Utilizing a safety factor of 1000 the ADI was set at 0.0025 mg/kg. This is equilivant to a MPI of 0.1500 mg/day for a 60 kg individual. The TMRC of Metolachlor in the daily diet based on the total tolerances above and a daily food intake of 1.5 kg is 0.07209 mg/day. Under these conditions 48.1 percent of the ADI has been utilized.

Reassesment of the dog study has resulted in the conclusion that the decrease in APTT observed may be compound-related. This conclusion does not effect the NOEL in the dog study.

Subsequently, a two-year feeding study in rats has been submitted (MRID 63398). The NOEL in this study is 30 ppm (1.5 mg/kg) based on an observation of testicular atropy in the males with a LEL of 300 ppm (15 mg/kg). Utilizing a safety factor of 100 the ADI may be set set at 0.015 mg/kg. This is equivilant to a MPI of 0.900 mg/day for a 60 kg individual. The TMRC of Metolachlor in the daily diet based on the total tolerances above and a daily food intake of 1.5 kg is 0.07209 mg/day. Under these conditions 8.01 percent of the ADI has been utilized.

D. Toxicological Issues

Oncogenicity in the rat

Two chronic rat feeding studies (MRID 15418 & 63398) on Metolachlor showed liver tumors in the females at the highest dose tested. The toxicological significance of these observations

has been the subject of a Peer Review by the Toxicology Branch. The following is abstracted from the report of that review (Engler 1985).

"Metolachlor was observed to produce primary liver tumors (neoplastic nodules plus hepatocellular carcinomas combined) in female rats at the highest dose level tested in two separate chronic feeding studies sponsored by the registrant, Ciba Giegy Corp."

"In the IBT rat study (No. 622-07925) the following incidence pattern of liver hyperplastic (i.e., neoplastic) nodules, cystic cholangiomas, carcinomas, and other tumors occurred in female rats receiving Metolachlor in the feed for 2 years.

Dose (ppm)	0	30	300	1000	3000
Number of Female Examined (final sacrifice)	54	58	60	60	60
Hypertrophic-Hyperplastic Nodules	1	1	3	3	9
Angiosarcoma	0	0	0	0	1
Cholangioma	0	0	1	0	0
Cystic Cholangioma	2	2	1	2	6
Carcinoma	0	0	0	0	2
Total (No. Animals with primary liver tumors)	3	3	5	5	15*

(*Three animals each bore two primary liver tumors.)

An increase in primary liver tumors was found in high dose female rats. In this study, hyperplastic nodules were included as an oncogenic response along with cystic cholangioma and carcinoma based on recommendations of the National Cancer Institute (Cancer Res. 35:32143223, 1975) and the National Academy of Science (J. NCI 64: No. 1, p. 185, 1980). This was the only oncogenic response observed in female rats. No statistically significant increase in the incidence of primary liver tumors was observed in male rats administered the same dose levels, although a slight positive trend was apparent. This IBT study was classified as "supplementary" data due to inadequate clinical chemistry determinations and dietary preparation records.

b) Repeat Hazelton-Raltech, Inc., Study: In this study (No. 80030) the following incidence pattern of liver neoplastic nodules and carcinomas occurred in female rats receiving Metolachlor in the feed for 2 years.

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Memo Engler, R., Peer Review of Metolachlor, Aug 23, 1985

Dose (ppm)	0	30	300	3000
No. Females	60	60	60	60
Neoplastic Nodules	0	1	2	6*
Carcinomas	0	0	0	1
Total No. animals with proliferative lesions	0	1	2	7**

* ($P < 0.05$) ** ($P < 0.01$)

A significantly increased incidence of proliferative hepatic lesions was found in high dose females at terminal sacrifice. The survival of the animals at 24 months was 54%, 57%, 42% and 57% for the control, low, mid and high dose groups. This was the only oncogenic response observed in female rats. No statistically significant increase in proliferative hepatic lesions was observed in male rats administered the same dose levels; however, there was a trend of increasing neoplastic nodules (1/60, 1/60, 0/60 and 4/60 at control, low, mid and high dose) in male rats but this was not the case for carcinomas (2/60, 1/60, 3/60 and 3/60 at control, low, mid and high doses) in males. When the incidence of these lesions was combined, no statistically significant effect was noted, although a trend was demonstrated (i.e. 3/60, 2/60, 3/60 and 7/60 at control, low, mid and high doses. This study was classified as "Core minimum".

Based on their evaluation of the evidence the Committee concluded;

"The Committee concluded that the data available for Metolachlor provides weak evidence of carcinogenicity. Before making a final conclusion on the oncogenic potential of Metolachlor, the Committee recommended that the registrant provide: (1) the full mutagenicity battery required by EPA; and (2) metabolism studies as required by the 1982 guidelines. Subsequent to receipt of this information, the Committee will reconvene to consider classification of the oncogenic potential of the chemical and possible recalculation of the Q-star (potency factor)."

As the data requirements identified by the Committee, metagenicity studies and a metabolism study, have also been identified by this Standard as data gaps, A final determination of the toxicological issue of Metolachlor oncogenicity cannot be made in this standard.

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TABLE A
GENERIC DATA REQUIREMENTS FOR METHOLACHLOR

ata Requirement	Composition 1/	Use Patterns 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?3/
158.135 Toxicology					
ACUTE TESTING:					
81-1 - Acute Oral - Rat	TGAI		Yes	15523	No
81-2 - Acute Dermal - Rabbit	TGAI		Yes	15526	No
81-3 - Acute Inhalation - Rat	TGAI		Yes	15535	No
81-4 - Eye Irritaion - Rabbit	TGAI		Yes	15526	No
81-5 - Dermal Irritation - Rabbit	TGAI		Yes	15530	No
81-6 - Dermal Sensitization - Guinea Pig	TGAI		Yes	15631	No
81-7 - Acute Delayed Neurotoxicity - Hen	TGAI		No		No4/
SUBCHRONIC TESTING:					
82-1 - 90-Day Feeding -					
Rat	TGAI		No		No5/
Dog	TGAI		Yes	17690 16632	No
82-2 - 21-Day Dermal-	TGAI		No		No6/
82-3 - 90-Day Dermal-	TGAI		No		No6/

TABLE A
GENERIC DATA REQUIREMENTS FOR METOLACHLOR

ta Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?3/
58.135 Toxicology (Cont.)					
82-4 - 90-Day Inhalation -	TGAI		No		No7/
82-5 - 90-Day Neurotoxicity-	TGAI		No		No8/
CHRONIC TESTING:					
83-1 - Chronic Toxicity -					
Rat	TGAI		Yes	63398	No
Non-rodent	TGAI				
83-2 - Oncogenicity Study -					
Rat	TGAI		Yes	15418 63398	No
Mouse	TGAI		Yes	84003 39194	No
83-3 - Teratogenicity -					
Rat	TGAI		Yes	15396	No
Rabbit	TGAI		Yes	41283	No
83-4 - Reproduction - Rat	TGAI		Yes	80897	No
MUTAGENICITY TESTING					
84-2 - Gene Mutation	TGAI		partially	15397	Yes9/ 9 months

TABLE A
GENERIC DATA REQUIREMENTS FOR METOLACHLOR

Data Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?3/	
158.135 Toxicology (continued)						
84-2 - Chromosomal Aberration	TGAI		partially	15630	Yes10/	12 months
84-2 - Other Mechanisms of Mutagenicity	TGAI		No		Yes	12 months
SPECIAL TESTING						
85-1 - General Metabolism	PAI or PAIRA		No		Yes	24 months
85 Effects on Coagulation	TGAI		No		Yes11/	

1/ Composition: TGAI Technical Grade Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient, Radiolabelled; Choice = Choice of several test substances determined on a case-by-case basis.
2/ The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Non-Food; C = Aquatic, Food Crop; D = Aquatic, Non-Food; E = Greenhouse, Food Crop; F = Greenhouse, Non-Food; G = Forestry; H = Domestic Outdoor; I = Indoor; IP = Industrial Preservative.
3/ Unless otherwise specified data must be submitted no later than six months after publication of this Standard
4/ Metolachlor is not a member of the chemical class, organophosphates, which must be subject to this test
5/ This requirement is waived based on the submission of an acceptable chronic feeding study in the rat
6/ This study is not required because existing acceptable end-uses should not result in repeated human skin contact
7/ This study is not required because existing acceptable end-uses should not result in repeated inhalation exposure
8/ This study is not required because the acute neurotoxicity study is not required
9/ Additional testing is required in an in vitro mammalian cell system
10/ Additional testing is required in a more sensitive test system
11/ The registrant is to devise studies on the effect of Metolachlor on coagulation in the dog. Protocols are to be provided within 6 months at which time the additional time necessary to perform the studies will be determined.

METOLACHLOR TOXICOLOGY BIBLIOGRAPHY

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15418 Gordon, D.E., (1978) Two-year chronic oral toxicity study - Albino rats, IBT No. 8532-07926. (Unpublished study received Jul 7, 1978 under 100-583; prepared by Industrial Bio-Test Laboratories Inc, submittedby Ciba-Geigy Corp, Greenboro NC CDL:097168-D)

15425 Hambock, H. (1974b) Project Report 12/74: Addendum to Project Report 7/74: Metabolism of CGA 24 705 in the Rat: AC 2.52; Received Nov. 25, 1975 under 6G1708. (Unpublished report prepared by Ciba-Geigy Ltd., Basle, Switzerland; CDL:94984-P)

15523 Bathe, R. (1973) Acute Oral LD_{50} of Technical CGA-24705 in the Rat: Project No. Siss 2979. Received Sep 26, 1974 under 5G1553. (Unpublished study prepared by Ciba-Geigy Corp., Ltd., Basle, Switzerland; CDL:112840-A)

15525 Roch, W.J., Emetic Dose 50 in Beagle Dogs with CGA-24705 Technical: Contract No. 120-2255-34. Received Sep 26, 1974, (Unpublished study prepared for Ciba-Geigy Corp., Greensboro, N.C.; CDL:112840-C)

15526 Bach, K.J., Acute Dermal LD_{50} of CGA-24705 - Technical in Rabbits: Contract No. 1202255-34. Received Sep 26, 1974 under 5G1553. (Unpublished study prepared for Ciba-Geigy Corp., Greensboro, N.C.; CDL:112840-E)

15527 Sachsse, K. (1973) Irritation of Technical CGA-24705 in the Rabbit Eye: Project No. Siss 2979. Received Sep 1974 under 5G1553. (Unpublished report prepared by Ciba-Geigy Ltd., Basle, Switzerland; CDL:112840-G)

15530 Sachsse, K. (1973) Skin Irritation in the Rabbit after Single Application of Technical CGA-24705: Project No. Siss 2979. Received Sep 26, 1974 under 5G1553. (Unpublished study prepared by Ciba-Geigy Ltd., Basle, Switzerland; CDL:112840 I)

15531 Sachsse, K. (1977) Skin Sensitizing (Contact Allergenic) Effect in Guinea Pigs of Technical CGA-24705. Project No. Siss 5726. Received October 17, 1977. [Unpublished study prepared by Ciba-Geigy Ltd., Basle, Switzerland]

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15630 Ciba-Geigy Limited (1976) Dominant Lethal Study on CGA 24705 Technical: Mouse (Test for Cytotoxic or Mutagenic Effects on Male Germinal Cells) PH 2.632. Received Jan 18, 1978 under 7F1913. (Unpublished study including Addendum; CDL:96717-C; 96717-D)

16632 Jessup, D.C.; Estes, F.L.; Jefferson, N.D.; et al. (1979) 6-month chronic oral toxicity study in beagle dogs: IRDC No 382-054. (Unpublished study including addendum and AG-A No. 5358, received Dec 11, 1979 under 100-597, prepared by International Research and Development Corp., submitted by Ciba-Gigy Corp. Greensboro, NC; CDL:099116-C)

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39194 Tisdel, M., Balk, M.W., Jackson, T., et al. (1980) Toxicity study metolachlor on mice, (Unpublished study no. 79020, receoed Jul 25, 1980, under 100-587, prepared by Hazleton Raltech Scientific Services and American College of Laboratory Animal Medicine, Submitted by Ciba- Geigy Corp, Greenboro NC CDL:242941

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80897 Smith, S.H., O'Loughlin, C.K., Salamon, C.M. et al., (1981) Two generation reproduction study in albino rts with metolachlor technical: Study No. 450-0272. Final rept. (Unpublished study received Sept 30, 1981 under 100-597; prepared by Whittaker Corp. submitted by Ciba-Geigy Corp., Greensboro NC; CDL: 245959-A; 245960; 245961)

84003 Marias, A.J.; Gesme, J.; Albanese, E.; et al. (1977) Revised final report to Chiba-Geigy Corporation: Carcinogenicity study with CGA-24705 technical in albino mice: IBT No. 622-07925 (8532-07925) (Unpublished study received Jul 7, 1978 under 100-583; prepared by Industrial Bio-Test Laboratories Inc; submitted to Ciba-Geigy Corp, Greensboro NC;CDL:097169-A 097170)

RZ001 Strasser, F. and Arni,P., Nucleous anomaly test in somatic interphase nuclei of chinese hamster. Ciba-Geigy Limited, Experimental Pathology Laboratory, Study No. 831498, October 1984

RZ002 Puri, E. and Muller, D., Autoradiographic DNA repair test on human fibroblasts. Ciba-Geigy Limited, Experimental Pathology Laboratory, Study No. 831499, November 1984

RZ003 Puri, E. and Muller, D., Autoradiographic DNA repair test on rat hepatocytes. Ciba-Geigy Limited, Experimental Pathology Laboratory, Study No. 831497, November 1984

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; Ciba Geigy;# 227625; 6/21/76	CGA 24705 technical		Teratogenic NOEL>360 mg/kg/day(HDT) Petotoxic NOEL > 360 mg/kg/day Maternal NOEL > 360 mg/kg/day Levels tested by gavage in Sprague - Dawley strain - 0, 60, 180 and 360 mg/kg/day		Minimum 000434
Teratology - rabbit; Argus Research Labs; #203-001; 7/25/80	Metolachlor (Technical) in hydroxymethyl cellulose	099630	Petotoxic NOEL > 360 mg/kg/day Teratogenic NOEL > 360 mg/kg Maternal NOEL = 120 mg/kg		Minimum 001051
3 Generation reproduction-rat; IBT; #622-07928; 1/4/78	CGA 24705 technical		NOEL > 1000 ppm (HDT) IBT Supplementary (1/31/80) Levels tested: 0,30, 300 & 1000 ppm.		Minimum 000434 Supplementary 000438 Supplementary 002265 Supplementary 003197
2-Generation reproduction - rat;Toxigenics, Inc.; # 450-0272; 08/31/81	TECH	245959 245960 245961	Reproductive NOEL = 300 ppm Reproductive LEL = 1000 ppm (reduced pup weights and reduced parental food consumption).		Guideline 001374
6 Month feeding - dog; IRDC; #382-054; 11/2/79	CGA 24705 technical		NOEL = 100 ppm LEL = 300 ppm(based on a lower rate of decrease for SAP levels) Levels tested = 0, 100, 300 and 1000 ppm in Beagles		Minimum 000432
90 Day feeding - rat; Oncins Research & Breeding Center;3/1/74	Metolachlor technical	112841	NOEL = 1000 ppm		Supplementary 000434 000436 000437 000438

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
90 Day feeding - dog; Oncins Research & Breeding Center;1974	CGA 24705 technical		NOEL = 500ppm		Minimum 000438 000436 000434
Oncogenic - mice; IBT;#622-07925; 12/15/77	CGA 24705 technical		Oncogenic NOEL = > 3000 ppm (HDT) IBT Valid (audited by Ciba-Geigy) Levels tested: 0,30,1000 & 3000 ppm.		Minimum 000434
2-Year feeding/oncogenic -rat; Hazleton Raltech; #80030; 5/2/83 & 4/29/85	Technical FL-800362	250369 250370 250371 250372 250373 250374 250375 245957 245958 258390	Oncogenic NOEL = 300 ppm Oncogenic LEL = 3000 ppm(Increased incidence of neoplastic nodules/hepatocellular carcinomas) Systemic NOEL = 30 ppm Systemic LEL = 300 ppm (testicular atrophy) Levels tested = 0, 30, 300, and 3000 ppm in CD-CRL: CD(SD)BR rats ADDENDUM Examination of nasal turbinates revealed additional evidence of weak oncogenicity at this site		Supplementary 001374 Supplementary 003435 Minimum 004199 Minimum 004200 Minimum 004725
2 Year feeding/onco-genic - rat; IBT; #622-07926; 2/9/79	Tech 99.9% Batch # FL-7502 27 & FL-752105	244166 099628 099626 070048	Systemic NOEL < 30 ppm (LDT) (decreased spleen wt) Oncogenic NOEL = 300 ppm Oncogenic LEL = 1000 ppm(heptocellu-lar carcinoma and cystic cholang-ioma) Dosage levels tested in Charles River strain - 0, 30, 300, 1000 and 3000 ppm. IBT - validated		Supplementary 001051

Tox Chem No. 188DD Metolachlor

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2 year feeding/oncogenic - mice; Hazleton Ral-tech; no. 79020; 8/13/82	Technical	248722	Oncogenic NOEL > 3000 ppm (HDT) Systemic NOEL = 1000 ppm Systemic LEL = 3000 ppm (decreased weight gain, decreased survival of females). Levels tested: 0, 300, 1000, and 3000 ppm		Minimum 003885 Minimum 004725
Mutagenic- dominant lethal- mice; Ciba Geigy; 9/8/76	CGA 24705 Technical		Negative mutagen		Minimum 000434
Laboratory audit - rat; oncogenic			This audit found no study deficiencies which would preclude this study from being classified as core minimum		004199
Acute oral LD_{50}-rat	CGA 24705 technical		LD_{50} = 2780mg/kg	III	000436 000428
Acute dermal LD_{50}-rabbit	CGA 24705 technical		LD_{50}>10,000 mg/kg	III	000428 000436
Primary eye irritation - rabbit	CGA 24705 technical		Draize score = 0/110		000436 000428
Primary dermal irritation -rabbit	CGA 24705 technical		PIS =0.1 not a primary irritant		000436 000428
Dermal sensitization - guinea pig	CGA 24705 technical		not a sensitizer		000436 000428
Acute oral - dog	CGA 24705 technical		LEL = 19 mg/kg emesis		000436

Tox Chem No. 188DD Metolachor

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute inhalation LC_{50}-rat	CGA 24705 technical		LC_{50} > 1, 750 mg/m^3/4 hours		000436 000428
Dermal sensitization - guinea pig; Ciba Geigy; 10/17/77	CGA 24705 technical		Positive reaction 16/20 tested		Minimum 000434
Acute oral LD_{50} - rat	CGA 24705 6EC		LD_{50}=4286 mg/kg (male) LD_{50}=2828 mg/kg (female)	III	000436
Acute oral - dog	CGA 24705 6EC		24.5 mg/kg - Emesis		000436
Acute dermal LD_{50} - rabbit	CGA 24705 6EC		LD_{50}>10,000 mg/kg	III	000436
Acute inhalation LC_{50} - rat	CGA 24705 6EC		LC_{50}>247 mg/L	IV	000436
Primary eye irritation-rabbit	CGA 24705 GEC		Scattered or diffuse areas of opacity persistent for 7 days	II	000436
Primary dermal irritation - rabbit	CGA 24705 GEC		non-irritating (1.6/8.0)	IV	000436
Acute oral LD_{50} - rat; IBT; #8530-10822; 10/28/77	Dual 8E		LD_{50}=2533.5 mg/kg (1888.5 -3398.9)	III	Minimum 000430
Acute dermal LD_{50} - rabbit;IBT;#8530-10822; 10/28/77	Dual 8E		LC_{50} >3038 mg/kg	III	Minimum 000430 000433
Acute inhalation LC_{50}-rat; IBT; #8562-10823; 10/28/77	Dual 8E		LC_{50} > 0.94 mg/L Invalid	II	000430 Acceptable

Tox Chem No. 18800 - Metolachlor

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation - rabbit;IBT#;8530-10822 ;10/21/77	Dual 8E		unwashed eyes - moderate corneal reversed in 7 days washed eyes - slight iris & moderate effects reversed in 3 days	II	minimum 000430
Primary dermal irritation - rabbit; IBT; # 8530-10822; 10/21/77	Dual 8E		moderate erythema & edema 2nd degree burns at 72 hrs	II II	minimum 000430
Acute oral LD_{50} - rat	Milocep		LD_{50} = 3868 mg/kg (male & female) (3142-4761) LD_{50} = 4811 (mg/kg (male) (3771-6139) LD_{50} = 2944 mg/kg (female) (2185-3965)	III	Minimum 000431
Acute dermal LD_{50} - rabbit;IRDC;#382-044; 10/17/78	Milocep		LD_{50}>5000 mg/kg	III	Minimum 000431
Primary eye irritation - rabbit;IRDC;# 382-045; 10/17/78	Milocep		Corneal opacity not reversed 7 days	I	Minimum 000431
Primary dermal irritation; rabbit; IRDC; #382-046; 10/17/76	Milocep		PIS = 2.0 at 72 hrs slight irritation	III	Minimum 000431
Acute inhalation LC_{50} - rat; IRDC;#382-047; 11/3/78	Milocep		LC_{50}>20.8 mg/L	IV	Minimum 000431
Acute oral LD_{50} - rat; IBT;# 601-07539; 11/7/75	Atrazine 51% CGA 24705 30.6%		LD_{50} = 4680 mg/kg	III	000429

Tox Chem No. 188DD - Metolachlor

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute dermal LD_{50} - rabbit; IBT;#601-07539; 11/7/75	Atrazine 51% CGA 24705 30.6%		LD>2000 mg/kg	III	000429
Primary eye irritation - rabbit; IBT; #601-07539; 11/7/75	Atrazine 51% CGA 274705 30.6%		Washed Eye - Max score = 6 at 24 hrs conjunctival irritation only Unwashed Eye - Max score = 37 at 48 Unwashed eye - max score = 37 at 48 hrs. - corneal opacity & vascularization. Severe irritant.	I	000429 000429
Primary dermal irritation - rabbit;- IBT; #601-07540; 11/7/75	Atrazine 51% CGA 24705		PIS = 2.3		000429
Acute inhalation LC_{50} - rat; IBT; #663-07540; 11/7/75	CGA 24705 & Atrazine		LC_{50} > 14,392 mg/m^3 Or /4.4 mg/L	III	000429
Acute oral LD_{50} - rat; Stillmeadow; #1355-79; 11/29/79	Metolachor 8E (formulation FL-790388)		LD_{50} = 4250 mg/kg (M) LD_{50} = 2700 mg/kg (F) Symptons: piloerection, ptosis exophthalmos and convulsions		
Acute dermal LD_{50} - rabbit; Stillmeadow; #1356-79; 10/23/79	Metolachlor 8E (formulation FL-790388)	242552	LD_{50} > 5031 mg/kg Erythema, edema, eschar formation and decreased activity.	III	Minimum 000849
Primary eye irritation - rabbit; Stillmeadow; #1252-79; 8/3/79	Metolachlor 8E (formulation FL-790388)	242552	[Test summaries and data differ substantially.]		Invalid 000849
Primary dermal irritation - rabbit; Stillmeadow; #1357-79; 10/5/79	Metolachlor 8E (formulation FL-790388)	242552	PIS = 5.21/8.0	II	Guideline 000849

Tox Chem No. 188DD - Metolachlor

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOK Category	CORE Grade/ Doc. No.
Acute oral LD_{50} - rat; Stillmeadow; #1352-79; 11/29/79	Metolachlor 8E (formulation FL-790393)	242553	LD_{50} = 4550 mg/kg (M) LD_{50} = 1680 mg/kg (F)	III	Guideline 000849
Acute dermal LD_{50} - rabbit; Stillmeadow; #1353-79; 10/23/79	Metolachlor 8E (formulation FL-790393)	242553	LD_{50} > 5010 mg/kg	III	Minimum 000849
Primary eye irritation - rabbit; Stillmeadow; #1253-79; 8/7/79	Metolachlor 8E (formulation FL-790393)	242553	No opacity; irritation persists 7 days.	II	Guideline 000849
Primary dermal irritation - rabbit; Stillmeadow; #1354-79; 10/5/79	Metolachlor 8E (formulation FL-790393)	242553	PIS = 4.3/8.0	III	Guideline 000849
Acute oral LD_{50} - rat; Stillmeadow; #1349-79; 11/21/79	Metolachlor 8E (formulation FL-790401)	242554	LD_{50} = 2690 mg/kg (M) LD_{50} = 820 mg/kg (F)	III	Guideline 000849
Acute dermal LD_{50} - rabbit; Stillmeadow; #1350-79; 10/25/79	Metolachlor 8E (formulation FL-790401)	242554	LD_{50} > 5009 mg/kg	III	Minimum 000849
Primary eye irritation - rabbit; Stillmeadow; #1165-79; 6/4/79	Metolachlor 8E (formulation FL-790401)	242554	[Test summaries and data differ significantly.]		Invalid 000849

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irritation - rabbit; Stillmeadow; #1351-79; 6/4/79	Metolachlor 8E (formulation FL-790401)	242554	PIS = 4.73/8.0	III	Guideline 000849
Acute oral LD_{50} - rat; Stillmeadow; #1346-79; 11/26/79	Metolachlor 8E (formulation FL-790403)	242555	LD_{50} = 2500 mg/kg (M) LD_{50} = 1250 mg/kg (F)	III	Guideline 000849
Acute dermal LD_{50} - rabbit; Stillmeadow; #1347-79; 10/23/79	Metolachlor 8E (formulation FL-790403)	242555	LD_{50} > 5008 mg/kg	III	Minimum 000849
Primary dermal irritation - rabbit; Stillmeadow; #1348-79; 10/5/79	Metolachlor 8E (formulation FL-790403)	242555	PIS = 4.65/8.0	III	Guideline 000849
Acute oral LD_{50} - rat; Stillmeadow Inc; #2307-81; 10/15/81	Metolachlor 15.0%	246884	LD_{50} greater than 5070 mg/kg Symptoms: dilated pupils, ptosis trigcid muscle tone	IV	Guideline 002797
Acute dermal LD_{50} - rabbit; Stillmeadow; #2308-81; 10/22/81	Metolachlor 15.0%	246884	LD_{50} greater than 2010 mg/kg no mortalities	III	Guideline 002797
Acute inhalation LC_{50}- rat; Toxigenics Inc; #420-0775; 12/22/81	Metolachlor 15.0%	246884	LC_{50} greater than 2.2 mg/L. no mortalities	III	Guideline 002797
Primary eye irritation- rabbit; Stillmeadow; #2309-81; 10/15/81	Metolachlor 15.0%	246884	At 24 hrs. 2/9 corneal opacity; 5/9 iris irritation;9/9 conjunctive irritation . corneal opacity and iris irritation had cleared by day 4. conjunctive irritation had cleared by day 7.	III	Guideline 002797

Tox Chem No: 1660D - Metolachlor

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irrit. - rabbit; Stillmeadow; #2310-81; 10/14/81	Metolachlor 15.0%	246884	At 24 hrs. 5/6 had slight erythema. no irritation at 72 hrs. PIS = 0.38	IV	Guideline 002797
Acute inhalation LC_{50} - rat; Toxigenics; #420-6048; 9/10/81	Metolachlor 15.0%	246376	LC_{50} greater than 2.73 mg/L (M) LC_{50} greater than 6.09 mg/L (F)	III	Guideline 002827
Acute oral LD_{50} - rat; Bioresearch Lab; #1682-D; 7/27/81	Atrazine 17.39% Atrazine related compounds ..... 0.91% Metolachlor 31.80%	246035	LD_{50} > 5 g/kg Lethargy, salivation, congested spleen, necrotic areas in the intestine.	IV	Guideline 002087
Acute dermal LD_{50} - rabbit; Bioresearch Lab; #1682-C; 7/20/81	Atrazine 17.39% Atrazine related compounds ..... 0.91% Metolachlor 31.80%	246035	LD_{50} > 2 g/kg	III	Guideline 002087
Acute inhalation LC_{50} - rat; Toxigenics; #420-0664; 7/28/81	Atrazine 17.39% Atrazine related compounds ..... 0.91% Metolachlor 31.80%	246035	LC_{50} > 2.34 mg/L	III	Guideline 002087
Primary eye irritation - rabbit; Bioresearch Lab; #1682-B; 7/20/81 7/20/81	Atrazine 17.39% Atrazine related compounds ..... 0.91% Metolachlor 31.80%	246035	At 24 hours, 4/6 corneal opacity 3/3 corneal opacity (1/6 = 5) (1/3 = 10, 1/3 = 20, 3/6 = 40 1/3 = 40) Conjunctive irritation present - all irritation had cleared by day 14 except for 1/6 animals had redness (1/6 = 1)	II	Guideline 002087

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irrit. - rabbit; Bioresearch Lab; #1682-A; 6/29/81	Atrazine 17.39% Atrazine related compounds ..... 0.91% Metolachlor 31.80%	246035	PIS = 0.21	IV	Guideline 002087
Dermal sensitization - guinea pig ; Bioresearch Lab; #1682-E; 8/31/81	Atrazine 17.39% Atrazine related compounds ..... 0.91% Metolachlor 31.80%	246035	Non sensitizing		Guideline 002087
Acute oral LD_{50} - rat; Int. Res. Dev. Corp.; 05/23/78	Banvel 4S + LASSO 4 EC	234450	LD_{50} > 5000 mg/kg	IV	Minimum 000045 000054
Acute dermal LD_{50} - rabbit; Int. Res. Dev. Corp.; 05/23/78	Banvel 4S + LASSO 4 EC	234450	LD_{50} > 20,000 mg/kg	IV	Minimum 000045 000054
Acute inhalation LC_{50} - rat; Int. Res. Dev. Corp.; 04/24/78	Banvel 4S + LASSO 4 EC	234450	LC_{50} > 22.2 mg/li (4 hours)	IV	Minimum 000045 000054
Primary eye irritation - rabbit; Int. Res. Dev. Corp.; 05/23/78	Banvel 4S + LASSO 4 EC	234450	Mild - conjunctivitis	IV	Guideline 000045 000054
Primary dermal irritation - rabbit; Int. Res. Dev. Corp.; 05/23/78	Banvel 4S + LASSO 4 EC	234450	Not irritating; PIS = 0.4/8	IV	Guideline 000045 000054

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic- micronucleus test - Chinese hamster; Ciba-Geigy; #831498; 10/84	Technical 95.9% a.i.	258390	No evidence of mutagenicity. No evidence that test material was absorbed or reached the target tissue, the bone marrow. Levels tested: 0, 1250, 2500 and 5000 mg/kg single dose by gavage in Chinese hamsters (strain unspec)		Inconclusive 004725
Mutagenic - Primary DNA Damage Assay - Fibroblasts; Ciba-Geigy, #831499; 11/84	Technical 95.9% a.i.	258390	No evidence of mutagenicity. Unacceptable because no rationale for the selection of doses was presented. Levels tested: 0.125, 0.625, 3.125, and 15.625 nl/ml in human fibroblasts (CRL 1121, ATCC).		Unacceptable 004725
Mutagenic - Primary DNA Damage Assay - Rat hepatocytes; Ciba-Geigy, #831497; 11/84	Technical 95.9% a.i.	258390	No evidence of mutagenicity. Unacceptable because no rationale for the selection of doses was presented. Levels tested: 0.25, 1.25, 6.25, and 31.25 nl/ml in hepatocytes isolated from adult male rat (Tif: RAIf[SPF]) Ciba-Geigy Tierfarm.		Unacceptable 004725
Risk assessment EPA					004200
Dissimilation chemicals, metabolites or impurity or contaminant or salt or photodegradent or etc			Caswell # 388AB		

Reviewed by Gary J. Burin, Toxicologist
Toxicology Branch, HED

Gary J. Burin 10/5/86
William S. Burnam 10/5/8

005504

Review of Data

1. Teratogenic Potential of CGA-24705 in New Zealand White Rabbits, performed at Argus Research Laboratories, Inc., Perkasie, Pa., July 25, 1980 and submitted by Ciba-Geigy Agricultural Division.

Sixty-four female New Zealand White rabbits were artificially inseminated with sperm from untreated, proven males from the same source and strain. Females were pretreated with Human Chorionic Gonadotropin prior to insemination.

Females were then randomly assigned to test groups which received either 0, 36, 120 or 360 mg/kg of CGA-24705 (95.4% pure) suspended in water with hydroxy methyl cellulose K 4M Premium (METHOCEL®) as the suspending agent. Animals received a volume of 10 ml/kg/day by gavage on days 6 through 18 of gestation based on body weight measurements which were made daily during the exposure period. Observations of clinical signs, abortions and delivery were made up to day 30 of gestation, at which time the does were killed by CO_2 asphyxiation and their uteri removed and examined. Fetuses and pups were then weighed and examined for visceral anomalies. Grossly observable visceral variations were removed, preserved with formalin and processed histologically. Finally, carcasses were eviscerated, stained with alizarin red S and examined for skeletal variations.

Results:

Maternal toxicity was evident in the high dose group in the form of lacrimation, miosis, decreased food consumption and decreased day 12 and 18 body weights. Of these signs of toxicity, only miosis was consistently found in the mid dose animals (one mid dose animal was reported to also have excess lacrimation). Thus, 360 mg/kg is the dose level in this study associated with frank maternal toxicity.

Two mortalities occurred in this study with one being found in the high dose group and one in the low dose group. Neither of the deaths were directly associated with the test compound although the intubation procedure and associated handling was likely to have been a precipitating factor in these deaths.

No compound-related effects were observed on litter size, numbers of early or late resorptions, fetal body weights, or frequency of variations among fetuses or pups. Among the specific variations observed, no compound related effects were evident. Although hydrocephalus with small exencephaly was observed in two fetuses from a dam treated with 360 mg/kg and was not seen in control, low or mid dose fetuses, the low incidence of this variation and the maternal toxicity seen in the dam which delivered those pups, suggest that it was not a true teratogenic response and that it may be either spontaneous in origin or associated with the maternal toxicity.

Core Classification

Core-Minimum. The NOELs for teratogenicity and fetal toxicity are 360 mg/kg. Frank maternal toxicity was observed only at the 360 mg/kg dose level.

Reviewed by Gary J. Burin, Toxicologist (s) Gary J. Burin 1/22/82
Toxicology Branch, HED

Two-Generation Reproduction Study with Metolachlor Technical in Albino Rats, conducted at Raltech Scientific Services and submitted by Ciba-Geigy on 9/30/81.

Metolachlor Technical was fed in the diet at dose levels of 0, 30, 300 or 1000 ppm to Charles River CD strain albino rats. Fifteen male and 30 females were assigned to each treatment group and were 32 days old when they first received test compound. Animals were mated after either 14 weeks (F_0) or 17 weeks (F_1) on test. Mating only occurred once per generation.

The F_1 parental animals were randomly selected from the F_{1a} litter after weaning of F_{1a}. F_0 males were sacrificed after 135 days on test and F_0 females were sacrificed after 164 days on test. Gross examination was conducted on all F_0 males and on F_0 females which displayed "untoward developmental anomalies". After 157 to 167 days on test, F_1 males were sacrificed and after 197 to 208 days, F_1 females were sacrificed. Gross and histological examinations were performed on all F_1 parents. Five randomly selected male and 5 female F_{1a} progeny in each dose group were also examined histologically.

The total number of pups, number delivered viable or stillborn and number found cannibalized were recorded for each litter. Pup survival to days 1, 4, 7, 14 and 21 after birth were recorded. Litters greater than 10 pups were randomly reduced in size on day 4. Pup body weights were recorded on days 4, 7, 14 and 21. Litters were observed daily.

Food consumption was measured weekly for each parental animal of the F_0 and F_1 generations only during the premating periods. Diet analyses were conducted on 15 separate occasions.

Organ weights for adrenal, brain, heart, kidneys, liver, spleen, testes and thyroid were recorded for all F_1 parental animals surviving to final sacrifice. The following tissues were examined histologically:

Adrenal	Pituitary
Aorta	Prostate
Bone (with marrow)	Salivary gland
Brain (3 levels)	Sciatic nerve
Esophagus	Skeletal muscle
Eyes	Skin
Heart	Small intestine
Kidneys	Spinal cord
Large intestines	Spleen
Liver (2 lobes)	Stomach
Lung	Testes
Lymph nodes	Trachea
Mammary gland	Thymus
Ovaries	Thyroid (with parathyroid)
Pancreas	Urinary bladder
	Uteri

In addition, all tissues appearing abormal were examined microscopically.

The study design was essentially as follows:

F_0 Generation Parental Animals

↓

Premating Period (14 weeks)

↓

Mating Trials (2 weeks)

↓

Gestation Periods (3 weeks)

↓

Lactation Periods (3 weeks)

↓ → Excess F_1 (sacrificed on lactation day 4)

F_1 Generation Parental Animals

↓

Premating Period (17 weeks)

↓

Mating Trials (2 weeks)

↓

Gestation Periods (3 weeks)

↓

Lactationm Periods (3 weeks)

↓

F_{2a} Weanlings

Results

Time weighed average concentrations of metolachlor, based on periodic diet analyses, were 0, 32.0, 294 and 959 ppm for the 0, 30, 300 and 1000 ppm groups, respectively. No deaths occurred among the F_0 animals or among the F_1 males. Two F_1 females were found dead during the premating period, one F_1 female was found dead on gestation day 19 and one F_1 female was sacrificed in moribund condition on lactation day 1; these animals belonged to the 300, 1000, 300 and 0 ppm groups respectively. No compound related effect on parental body weight was apparent. Food consumption was not effected by treatment in the F_0 generation but was significantly reduced for the F_1 30 ppm females at week 16, 300 ppm females at weeks 6, 7 and 10 and the 1000 ppm females at weeks 1, 6, 7, 8, 10, 12, 13 and 15, as compared to controls.

Clinical observations of parental animals did not indicate effects which could be associated with treatment. The mating, gestation, lactation, female fertility and male fertility indices did not appear to be effected by treatment in either generation. Pup survival was also not effected by treatment.

Pup body weights of the 1000 ppm dose level group were significantly reduced for the F_{1a} litters on days 14 and 21 and on days 4, 7, 14 and 21 for the F_{2a} litters. Pup body weights of the 30 and 300 ppm dose groups did not appear to be effected in a compound-related manner.

The incidence of external anomalies observed in pups did not appear to be effected by treatment. Gross and histological examination of parents and progeny did not reveal any lesions which appeared to be related to treatment.

Liver to body weight ratios were significantly increased for both F_1 parental males and females at the 1000 ppm dose level. The thyroid to body weight ratio and the thyroid to brain weight ratio of 1000 ppm F_1 males were significantly increased. Other organ weight comparisons that were statistically significant compared to controls did not appear to be related to treatment. Body weights of the weanling 1000 ppm F_{1a} females and F_{2a} males were reduced, though not significantly, and body weights of F_{2a} weanling females were significantly reduced for the 1000 ppm dose level.

Core Classification

Core Guidelines. The NOEL for reproductive effects is 300 ppm based on reduced pup weights and reduced parental food consumption at 1000 ppm. Other effects that may be related to treatment were increased liver to body weight and thyroid to body weight ratios in the 1000 ppm F_1 parents.

005504

003885

Study: Carcinogenicty Study With Metolachlor in Albino Mice

Accession No.: 248722

Sponsor/Contracting Lab.: Ciba-Geigy/Hazelton Raltech (Madison, WI)

Study No.: 79020

Report Date/Submitted: 8-13-82/10-2-82

Reviewer: D. Stephen Saunders Jr., Ph.D. DSS 7/30/84

Methods

The methods from the submitted study have been photocopied and are appended. The procedure followed in this study is unremarkable except for the following point:

1) Method of sacrifice of animals not described.

Test Compound

Metolachlor technical, batch no. FL-791174. % a.i. not disclosed in the final report, however it was stated that purity was determined by the sponsor prior to study initiation and at 3-month intervals thereafter. These data are on file with the sponsor. PM team 23 provided a value of 95.0% for the technical material (personal communication).

Results

A. Test diet analysis- Samples of each test diet for weeks 1-4 were analyzed for content of metolachlor. Thereafter, one diet was selected at random each week for analysis of content of the test material. Time-weighted averages of the three test diets indicated that all diets were within 5% of theoretical:

Diet (ppm)	Time-weighted Average (ppm)[a]	Time-weighted %Theoretical
300 (range)	287 (146-351)	96%
1000 (range)	981 (781-1120)	98%
3000 (range)	3087 (2660-3270)	103%

[a]data excerpted from submitted study.

B. Physical signs and Mortality- No significant treatment-related signs were noted. A slight increase in the overall incidence of signs related to the eye were noted as a result of treatment, however several distinct observations, including conjuctivitis, "eyes red", "eyes opaque", and "exudate from

eye", were counted together. No single physical sign was noted in increased frequency that could be related to treatment.

The only group which exhibited a mortality rate that was significantly higher than control or other treatment groups was the high dose females (group 8). This result was considered to be due to a number of deaths in the first weeks of the study that were the result of infection with Sendai virus. If these deaths were factored out of the analysis, no statistically significant differences in mortality existed between any of the groups. For the purpose of this review, these deaths have been considered to be treatment-related: animals in all groups were housed in the same room, and were exposed to the same environment. Since increased susceptibility to infection as a result of exposure to toxic substances is a recognized toxicological endpoint, removal of these deaths from the data base is not considered appropriate by this reviewer.

Dates of death for control and high dose males and females (groups 1, 4, 5, and 8) listed in table 3 of the final report were checked by this reviewer against individual animal pathology sheets, a.d were accurate. Relative survival was calculated for these groups by the reviewer; one minor error was found (animal #5083 died on test, counted as terminal sacrifice).

Relative survival for all groups is presented below in table 1.

Table 1. Relative Survival[a]

Dose (ppm)	Week 79		Week 105	
	Male	Female	Male	Female
0	41/52[b] (78.8)[c]	44/52 (84.6)	20/52 (38.5)	28/52 (53.8)
300	42/52 (80.8)	37/52 (71.2)	25/52 (48.1)	20/52 (38.5)
1000	43/52 (82.7)	40/52 (76.9)	31/52 (59.6)	24/52 (46.2)
3000	37/52 (71.2)	31/52 (59.6)	28/52 (53.8)	18/52* (34.6)

[a]data excerpted from submitted study.

[b]number alive/total. Total does not include 8 animals/group sacrificed at 12 and 18 months.

[c]percent, calculated by reviewer.

*$p<0.05$

C. Body Weight- Statistically significant reductions in body weight gain were observed for high dose male and female mice. Significant reductions in weight gain were noted for high dose males (group 4) after two weeks of treatment, and this deficit persisted throughout treatment. High dose females (group 8) had significant weight gain deficits beginning with week 32, and at

23/37 time points measured after this time statistically signfficant deficits were observed.

Average body weights were recalculated by this reviewer from submitted individual animal data for groups 1, 4, 5, and 8 on weeks 50 and 104; no errors were found.

Body weight data are presented in table 2.

Table 2. Effect of Metolachlor on Body Weight[a]

Dose (ppm)	Week 50 Male	Week 50 Female	Week 104 Male	Week 104 Female
0	40.3±4.1[b]	31.7±4.1	40.5±3.4	35.2±3.8
300	39.8±5.2 (98.8)[c]	31.7±2.9 (100.0)	40.9±4.3 (101.0)	34.3±6.1 (97.4)
1000	39.5±4.6 (98.0)	31.7±2.6 (100.0)	39.7±4.1 (98.0)	34.7±4.6 (98.6)
3000	36.5±3.2** (90.6)	30.3±2.7* (95.6)	37.9±3.6** (93.6)	32.6±3.6 (92.6)

[a]data excerpted from submitted study.

[b]body weight in grams, mean ± std. dev., calculated by reviewer from submitted individual animal data.

[c]percent of control, calculated by reviewer.

*p<0.05, **p<0.01 by Dunnett's t-test.

D. Feed Consumption and Compound Intake- No differences in food intake were noted between male treatment groups until week 90 of treatment, at which time high dose males ate about 10% less than control. This difference was statistically significant on weeks 98, 102 and 104. No significant effect on food consumption was noted betweem any of the female treatment groups. However, females tended to eat more food than their male counterparts.

Average food consumption for high dose and control male and female mice was calculated by the reviewer from submitted raw data for weeks 50 and 104 and compared to submitted summary data; no errors were found.

Compound intake was calculated by the reviewer based on average food intake and average body weights on weeks 26, 52, 78 and 104. All groups tended to consume less test compound (based on mg/kg body weight) in the latter portion of the study. Based on these calculations, female mice are estimated to have received a dose of metolachlor that was about 15-50% higher than corresponding males. This effect was due to the higher apparent food consumption for females coupled with the lower body weights for females compared to males. Since the effect of the test compound on body weight gain was similar in male and female

mice, the calculated difference in estimated compound intake is not considered significant.

Table 3 presents the calculated doses of test compound.

Table 3. Calculated Dose of Test Compound[a]

	Group	Diet (ppm)	Week 26	52	78	104
Males	2	300	54[b]	53	46	46
	3	1000	174	185	169	153
	4	3000	539	568	575	421
Females	6	300	65	77	61	54
	7	1000	239	253	226	177
	8	3000	703	852	655	607

[a]data excerpted from submitted study.

[b]dose of metoloachlor in mg/kg body weight, calculated by reviewer based on average food consumption and average body weights.

E. Clinical Pathology- No toxicologically significant effects on hematology, serum chemistries, or urinalyses were noted as a result of treatment with the test compound in any of the treatment groups.

(1) Hematology- An increase in white blood count was observed for group 2 (300 ppm males) at 18 months, however this result was due to a very high value for one animal (out of 8) (#5171, 78.8 x $10^3/mm^3$). This effect was not repeated at other time points nor was it dose-related. A statistically significant increase in the %neutrophils was also observed at 18 months for group 4 (high dose males). However, this increase was not accompanied by an increase in the WBC count, and, although the increase was statistically significant when compared to concurrent study controls, the values were within the range for normal CD-1 mice (ref. "Representative Historical Control Data", Feb. 1984, Hazelton Laboratories America, Inc.). Other hematology values were not altered.

(2) Serum Chemistries- An increase in average values for AST and ALT was noted at 24 months in high dose males (615.4 ± 901.0 and 306.2 ± 575.7, N = 6, AST and ALT respectively). The increases in average values were due to one animal with abnormally high values (#5275, AST = 2450.6, ALT = 1481.1 IU/L), as reflected by the large standard deviations for the averages. If these values were excluded, the averages were not different from control (AST = 248.3 ± 65.9, ALT = 71.2 ± 14.6; N = 5) and were within the normal range for CD-1 mice (see ref. above).

High dose females (group 8) also had a statistically significant increase in the average for serum AST activity and a decrease in serum uric acid content, both at 12 months. Two animals in the sample had values substantially higher than the other 5 animals in the group, as is reflected by the large standard deviation for the average (414.4 ± 258.0, N = 7). However, the average AST activity without the two high values was still significantly higher than control (267.7 ± 73.6, N = 5, vs. 168.5 ± 69.0, N = 6), and each of the individual values for this group were higher than the average control value. Therefore, even though average AST activity for high dose females was similar to control at 18 and 24 months, the increased activity at 12 months was likely treatment-related. Similarly, the decrease in serum uric acid content in this group at the 12 month interim sacrifice could not be attributed to the influence of out-lying values, and was likely treatment-related.

An approximate two-fold increase in average serum alkaline phosphatase activity was noted in all male treatment groups (groups 2-4) at 24 months. In each group, one animal with an abnormally high value (of 6 or 7 animals per group for which this value was determined) was responsible for the increase in the average. This effect was not dose-related, and only one animal in each group was a responder.

Other serum chemistry values were unremarkable.

(3) Urinalysis- Alterations in average values for protein content were observed, however in each case the increased average could be attributed to the influence of out-lying values. No trends in terms of dose or time-course were apparent. No notable alterations in other parameters were observed.

F. Organ Weights- Statistically significant changes in absolute and organ/body weight ratios were occassionally noted in response to treatment with the test compound. However, organ/brain weight ratios were not significantly altered in any of the treatment groups at any time point. For example, high dose males had statistically significant increases in liver and kidney organ/body weight ratios at 12, 18 and 24 months, and a decrease in the organ/body weight ratio of seminal vesicle at 24 months. These effects could be attributed to decreases in body weight rather than effects on the organs, with the exception of seminal vesicle which had an organ/brain weight ratio that was 55% of control but not statistically significant.

Similarly, effects on the absolute weights and/or organ/body weight ratios were noted in other organs such as kidney, ovaries and uterus, however statistically significant changes in organ/brain weight ratios were not seen in these tissues.

Organ weights for control and high dose male and female rats that were listed in the raw data summaries were compared by the reviewer to the hand-written values that were recorded on individual animal pathology sheets at sacrifice; all values appeared to be recorded accurately. Organ weight ratios were spot-checked, and appeared to have been calculated correctly.

G. Necropsy Data- (1) Gross findings: No significant treatment-related findings were noted upon macroscopic examination of animals at necropsy. Frequent findings included cortical cysts in the kidneys, enlarged uterus, cystic ovaries, and enlarged seminal vesicles. Other occasional findings included abnormal color or focus in the lung, and abnormal color and/or nodules or masses in the liver. None of these changes occurred in a manner that would suggest a dose-effect relationship with the test compound. There was no significant difference in the distribution of gross observations between animals necropsied at scheduled sacrifice and those that died on test or were sacrificed moribund.

Tabulated summaries of gross findings were compared to individual animal pathology sheets for the 12 and 18 month interim sacrifices; all tabulations appeared accurate. Findings of interest were spot-checked for animals that died on test (including moribund sacrifice) and for final (24 month) sacrifice, and were accurately recorded and tabulated.

Tabulations of gross lesions and resultant histological diagnoses were checked for lung and liver lesions for all treatment groups against individual animal pathology sheets, and were accurately recorded.

(2) Microscopic- Neoplastic lesions seen in all treatment and control groups included alveologenic tumor, nodular hyperplasia/hepatocellular carcinoma, and lymphosarcoma. No dose-related trends were apparent for any of these lesions when all histopathology data were considered.

The incidences of nodular hyperplasia/hepatocellular carcinoma and lymphosarcoma/reticulum cell sarcoma are depicted in table 6.

An apparent increase in the incidence of alveologenic tumor was observed in male mice at the 18 month interim sacrifice. The difference between group 1 control (0/8) and group 4 high dose (5/8) mice was suggestive of a positive response, and the trend was statistically significant by the method of Peto ($p = 0.02$) and by Fisher's Exact test ($p = 0.02$, see appendix 2). Although suggestive of an effect at 18 months, these data were not confirmed at final (24 month) sacrifice, when the incidences for control (5/20, 25%) and high dose (10/28, 35.7%) males were not significantly different. Addition of data from animals that were sacrificed moribund or died on test also indicated that the data obtained at 18 months were spurious, as evidenced by the lack of a dose-effect relationship for the total incidence of this lesion (table 5). Therefore, the apparent response at 18 months is considered artifactual and of no toxicological significance.

The incidence of alveologenic tumors for all animals (interim and final sacrifices and died on test/moribund sacrifice) is presented in table 5.

Commonly observed non-neoplastic lesions included cystic ovaries and endometrial hyperplasia in females, and lymphoid infiltration and cortical cysts of the kidney in both sexes. The incidences of these and other lesions were not dose-related.

(3) Correlation between gross and histological observations- Observations recorded at necropsy were compared to microscopic findings and tabulated by the investigators. A number of gross findings at necropsy, principally in the liver, kidney and lymph nodes, had no corresponding microscopic diagnosis and were listed as "not remarkable". Because only positive findings were recorded on the individual animal pathology sheets, it was not possible for this reviewer to independently verify that these gross lesions were actually examined microscopically. However, a tissue inventory was present with each individual animal pathology sheet which indicated the tissues present on each slide. Also, occassional recuts were requested by the study pathologists, apparently in order to locate lesions that were not present on the original slide. Two lung nodules were noted on gross necropsy that were listed as "not remarkable" on microscopic examinations (#5326, group 5, and #5552, group 8; both at final sacrifice). Neither of these nodules, even if they were re-examined and diagnosed as tumors, would change the interpretation of this study.

The remainder of the missing diagnoses were for abnormal color or size of tissues noted at necropsy, with the exception of kidney which included a number of tissues with cortical cysts that were not observed microscopically. For liver, spleen and lymph nodes, the investigators stated in the final report that these tissues "were frequently normal when examined microscopically".

In the case of kidney, the investigators stated that "there was not a good correlation between abnormal observations ... and the corresponding microscopic diagnoses". Most of these disparities were for cortical cysts, which were observed at necropsy, but apparently did not appear on the slide for microscopic examination. Since cortical cysts can be detected by gross observation, and no treatment-related effect on the incidence of this finding was noted, the lack of correlation for this particular lesion is not considered significant.

Table 5. Incidence of Alveologenic Tumors- Males[a]

Group (Dose)	Interim 12 mos.	Interim 18 mos.	Final 24 mos.	Died on test/ Moribund Sac.	Total
1 (0 ppm)	1/8[b] (12.5%)	0/8 -	5/20 (25.0%)	5/28 (17.9%)	11/64 (17.2%)
2 (300 ppm)	1/8 (12.5%)	4/8 (50.0%)	11/25 (44.0%)	6/21 (28.6%)	22/62 (35.5%)
3 (1000 ppm)	0/8 -	2/8 (25.0%)	5/29 (17.2%)	1/20 (5.0%)	8/65 (12.3%)
4 (3000 ppm)	0/8 -	5/8 (62.5%)	10/28 (35.7%)	4/21 (19.0%)	19/65 (27.9%)

(con't)

Table 5. Incidence of Alveologenic Tumors- Females[a]

Group (Dose)	Interim 12 mos.	Interim 18 mos.	Final 24 mos.	Died on test/ Moribund Sac.	Total
5 (0 ppm)	1/8 (12.5%)	2/8 (25.0%)	6/26 (23.1%)	6/25 (23.1%)	15/67 (22.4%)
6 (300 ppm)	1/8 (12.5%)	1/8 (12.5%)	8/20 (40.0%)	5/30 (16.7%)	15/66 (22.7%)
7 (1000 ppm)	0/8 -	4/8 (50.0%)	10/23 (43.5%)	3/28 (10.7%)	17/67 (25.4%)
8 (3000 ppm)	0/8 -	3/8 (37.5%)	4/17 (25.5%)	2/33 (6.1%)	9/66 (13.6%)

[a]data excerpted from submitted study.

[b]number of tumors/number of animals examined.

Table 6. Incidences of Liver and Lymphoid Tumors[a]

	Males				Females			
	Dose (ppm)							
Lesion	0	300	1000	3000	0	300	1000	3000
Nodular hyperplasia	7	8	12	8	1	2	2	2
Hepatocellular carc.	2	0	4	1	1	0	0	0
Total/no. examined	9/63	8/64	16/65	9/64	2/66	2/65	2/65	2/66
Lymphoid Neoplasias[c]								
-lung	2/64[b]	5/62	2/65	1/65	7/67	6/66	2/67	6/66
-spleen	3/60	3/63	3/64	0/64	7/66	6/66	4/66	7/66
-liver	4/63	4/64	3/65	0/64	6/66	5/65	5/65	7/66
-kidney	5/64	4/63	2/64	0/65	5/66	5/66	4/68	6/66
-mesenteric l.n.	5/58	4/62	3/61	1/63	8/65	4/63	5/63	8/64
no. affected animals	5	5	3	1	11	7	7	12

[a]data excerpted from table 46 of submitted study.

[b]number affected/number examined.

[c]includes lymphosarcoma and reticulum cell sarcoma.

Conclusions

Treatment of mice for 24 months with diets containing 300, 1000 or 3000 ppm of metolachlor failed to produce an increase in tumor incidence. A statistically significant increase in the incidence of alveologenic tumors in males was noted at the 18 month interim sacrifice, however this effect was not confirmed by the 24 month final sacrifice nor by total incidences for all animals. Other neoplastic lesions of the liver and lymphoid system were observed, however were not dose-related.

Animals of the high dose group gained significantly less body weight than did control animals, indicating that the high dose was an MTD.

Effects on organ/body weight ratios were observed in response to treatment with the test compound, particularly in the liver, kidney and ovaries. Although these alterations were statistically significant, similar effects on organ/brain weight ratios were not observed, and no lesions were detected in these organs upon gross and histological examination to suggest a pathogenic process that was dose-related.

Classification: Core-Minimum Method of sacrifice not described; purity of test article not disclosed although report states that purity of the test article was determined by the registrant prior to study initiation and at 3-month intervals during the study.

Not a carcinogen at the HDT (3000 ppm).

Systemic NOEL: 1000 ppm
Systemic LEL: 3000 ppm decreased body weight gain, decreased survival of high dose females.

Appendix 1. METHODS

INTRODUCTION

A study to determine the oncogenic potential of metolachlor in albino mice was conducted at the request of CIBA-GEIGY Corporation, Agricultural Division, Greensboro, North Carolina. The study, initiated on September 6, 1979 and terminated on September 8-11, 1981, followed the amended protocol of August 13, 1979. No protocol deviations (listed in Appendix A) influencing the quality of the study or interpretation of the data occurred during the conduct of the study.

This is the Final Report on Study No. 79020. It presents a description of the test material and the test system, procedures followed, all data collected, and relevant discussions within the six volumes of this report. Pages are numbered consecutively from the first page of the narrative volume to the last page of Appendix E.

TEST MATERIAL

Source and Identification

The test material (approximately 1.5-kg, sample and container) was received from CIBA-GEIGY on August 9, 1979, with the following label information:

Generic Name: Metolachlor Technical
Batch Number: FL-791174

For internal use, it was assigned Baltech Sample No. 744057. All material used in this study was drawn from this sample.

Purity and Stability

The purity of the test material was determined by CIBA-GEIGY prior to study initiation. In addition, 1-g samples of the test material were returned to CIBA-GEIGY for analysis at approximately 3-month intervals during the study to ensure purity and stability over the life of the study. These data are on file with CIBA-GEIGY.

Storage

The sample of metolachlor was refrigerated (4°F) throughout the study period. Test diets were stored at room temperature.

Safety Precautions

Personnel working with the test material or the test diets and those working in the animal room wore disposable masks, gloves, shoe covers, bonnets, and other appropriate clothing such as lab coats and uniforms.

Disposal

All waste feed, solid animal wastes, cage boards, and animal carcasses resulting from this study were disposed of in a high-temperature incinerator (U.S. Smelting Furnace Company, Belleville, Illinois). A 50-g sample of the test material was retained at Hazleton Raltech.

TEST SYSTEM

Animal Model

Charles River CD-1 albino mice, Crl:CD®-1(ICR)BR, purchased from the Portage, Michigan facility of Charles River Breeding Laboratories, Wilmington, Massachusetts, were received on August 29, 1979, (Charles River Order No. 2091703). The animals were immediately taken to Room 307 where they were acclimated for approximately 1 week prior to being placed on test. The CD-1 mouse is commonly used for oncogenicity studies in rodents, and Hazleton Raltech has adequate historical data for making appropriate comparisons.

Housing and Maintenance

Animals were individually housed in stainless steel, screen-bottom cages, 5 7/8 in. x 8 in. x 5 1/8 in., up to 140 per rack, held in a dual-corridor (access/return) room (Room 307), which was dedicated solely to this study. The following environmental conditions were maintained: 12 to 15 changes of 100% filtered outside air, 72°F ± 3°, 30-70% relative humidity, an automatically timed 12-hour light/12-hour dark cycle. Temperature and humidity were monitored continuously by a JC80 computer (Johnson Controls, Madison, Wisconsin), and deviations were recorded. Cage boards (DACP®) were changed at least twice weekly and animals were transferred to clean cages a minimum of every 2 weeks. Racks were repositioned within the room every 2 weeks to minimize the effect of any environmental variations. Care was taken to ensure that the animals were not disturbed for reasons other than routine maintenance and data collection.

During acclimation and through 4 days on test, all mice were on a redundant watering system; i.e., water bottles and automatic watering system (Systems Engineering, Napa, California). Three days after water bottles were removed, Week 1 body weights revealed that many animals had lost weight from the Week 0 (initiation) weight. Subsequent investigation revealed that many of the water valves were not functioning, or in some cases the mice had apparently not

learned to use the valves. Water bottles were replaced and the automatic waterers disconnected. For the remainder of the study, mice received fresh tap water twice weekly from clean, 2-oz, clear glass bottles equipped with rubber stoppers and stainless steel sipper tubes. Fresh feed was provided ad libitum on a weekly basis in clean, clear glass jars that allow easy inspection of the amount and condition of the feed. Uneaten feed was discarded. Water analyses provided by the City of Madison are on file at Hazleton Raltech.

Conditions for animals in this study were consistent with those in the ILAR Guide for the Care and Use of Laboratory Animals.

Identification

Each animal selected for study was assigned a unique eight-digit identification number and was permanently identified with a metal tag which was engraved with this number and attached to the loose skin at the back of the neck. Each animal's cage was also marked with its number. If an animal lost its tag, it was retagged when it did not have a cutaneous lesion in the neck tag region. All data collected from an animal were recorded under its identification number.

Prestudy Health Evaluation and Quality Control

During the acclimation period, five males and five females from mice purchased for this study were selected for diagnostic quality control health examinations. Results were negative for endo- and ectoparasites, for pathogenic lung and colon bacteria, for mycoplasma, and for all serological tests performed. An additional 10 males and 10 females were selected and bled for prestudy hematologic evaluation. Hematology parameters from all animals were normal.

Randomization

Mice were assigned at random to groups using a random numbers table (Reference: Planen und Auswerten von Versuchen, Birkhaeüser, Basel, 1953, p. 177, ed. seq. p. 131). Holding cages were numbered 1 to n for each sex, then 68 animals were selected at random for each group.

Vertical double rows (14 cages) in the study racks were numbered 1-20 for each sex, then each test group was randomly assigned to 5 double rows (4 double rows of 14 and 1 double row of 12 = 68 cages per group).

PROCEDURES

Route of Test Material Administration

Since the potential long-term human exposure to the test material would be oral, the test compound was administered orally by incorporation into the basal diet and ad libitum feeding.

Basal Diet

Basal diet for this study was Purina Certified Rodent Chow® #5002, identified by lot number and recorded. This feed is analyzed by the manufacturer for nutritional components and environmental contaminants prior to its release. The maximum concentrations of the contaminants are certified. No components or contaminants known to be in the basal diet could be expected to have interfered with the study.

Dose Levels

Four test diets were fed ad libitum throughout the study: three constant dietary concentrations of metolachlor technical and one diet containing no metolachlor technical. The metolachlor technical was added to the diet at the following concentrations:

Negative Control	0 ppm Metolachlor Technical
Low Level	300 ppm Metolachlor Technical
Mid Level	1000 ppm Metolachlor Technical
High Level	3000 ppm Metolachlor Technical

Diet Preparation and Storage

Test diets for the first 18 weeks of the study were prepared by mixing one part metolachlor and two parts ethanol, then totally admixing with the correct amount of basal diet to yield a premix containing 1% metolachlor. Finished test diets were then formulated from the appropriate amounts of 1% metolachlor premix and additional basal diet to achieve the desired metolachlor concentrations. The control diet was also mixed with ethanol.

Because of the concern for possible effects from the small amount of ethanol which remained in the diet after mixing, the ethanol was eliminated from the diets beginning in Week 19. The metolachlor was added directly to a small amount of basal diet. This was then mixed with enough additional basal diet for the finished diet. The mixing of finished test diets did not change. ethanol was also eliminated from the control diet.

iets were mixed fresh weekly throughout the study and stored in covered olyethylene containers at room temperature. Feed remaining in these ontainers at the end of the week was discarded.

Analysis of Test Diets

Test diets were assayed for metolachlor concentration to show that mixing procedures resulted in relatively homogeneous diets, that the test material was stable in the basal diet over the feeding period and until the assay was conducted, and that animals were being fed the proper dietary levels of metolachlor.

Assays for homogeneity were done on samples taken from the top, bottom, and two opposing sides of the mixing bowl for the control low and high level diets. The results served as the initial concentrations of these diets which were subsequently assayed after being held for 1, 2, 3, and 4 weeks at room temperature to show metolachlor stability.

During Weeks 1-4, all test diets were assayed, then during Weeks 5-104, one diet per week was selected at random from the three metolachlor test diets and assayed.

Additionally, all diets from Weeks 52, 73, 85, 97, and 101 were sent to CIBA-GEIGY for confirmatory analysis. These data are on file with CIBA-GEIGY.

Study Design

From over 544 mice, 68 mice of each sex were assigned at random to test and control groups with the following design.

Group	Animal Numbers*	Number of Mice	Sex	Treatment - Metolachlor (ppm)
1	61905041-61905108	68	M	Negative Control - 0 ppm
2	61905111-61905178	68	M	Low Level - 300 ppm
3	61905181-61905248	68	M	Mid Level - 1000 ppm
4	61905251-61905319	68	M	High Level - 3000 ppm
5	61905321-61905388	68	F	Negative Control - 0 ppm
6	61905391-61905458	68	F	Low Level - 300 ppm
7	61905461-61905528	68	F	Mid Level - 1000 ppm
8	61905531-61905598	68	F	High Level - 3000 ppm
-	61905601-61905610	10	M	For prestudy hematology
-	61905611-61905620	10	F	For prestudy hematology

*For convenience, the first animal in each of Groups 1-8 was assigned a number with the last digit 1. Animal No. 61905281 died on Day 5 and was replaced with Animal No. 61905319. Tissues from 61905281 were processed and read, but data was not included in summaries.

Treatment Duration and Study Termination

Each animal received its test diet *ad libitum* throughout the entire study period from initiation (September 6, 1979) through terminal sacrifice after 104 weeks on test (September 8-11, 1981), unless it died or was sacrificed *in extremis* prior to terminal sacrifice or was sacrificed after 12 or 18 months on test for clinical laboratory studies.

Antemortem Observations

Each animal was observed twice daily (a.m. and p.m.) for moribundity, mortality, or obvious signs of toxicity by an appropriately trained observer. At least once each week each animal was removed from its cage and carefully examined for abnormal appearance or behavior. Beginning during Week 14, the weekly observation included palpation for tissue masses. A notation was made weekly for each animal regardless of condition.

Body Weights and Feed Consumptions

Individual body weights were recorded for all animals at initiation, weekly through Week 13, then every second week beginning at Week 16. Individual weekly feed consumptions were recorded for the same 10 animals selected at random prior to initiation from each group for Weeks 1-13, then every second week beginning Week 16. If an animal selected for feed consumption data collection died or was sacrificed in extremis, it was replaced by random selection from the survivors in that group.

Clinical Laboratory Studies

Clinical laboratory studies including hematology, serum chemistry, and urinalysis were conducted according to Standard Operating Procedures on eight animals selected at random from the survivors in each group (16 per test diet) after 12 months and 18 months on test and at termination after 24 months on test. An additional eight animals per group were selected at termination for measurement of additional serum chemistry parameters.

Blood was obtained from the orbital sinus of mice fasted overnight. Urine was collected overnight from mice held in metabolism cages. All mice selected for clinical laboratory studies were necropsied after collection of samples.

The following hematology parameters, or as many as the available whole blood sample would permit, were measured:

Parameter

Total erythrocyte count
Total leucocyte count
Differential leucocyte count
Hematocrit
Hemoglobin
Platelet count

When hematologic evidence of anemia was present:

Reticulocyte count
Heinz body determination

The following serum chemistry parameters, or as many as available serum sample would permit, were measured:

Parameter

Alkaline phosphatase
BUN
Fasting glucose
ALT (aka SGPT)
Total protein*
Total cholesterol
Inorganic phosphorus
AST (aka SGOT)
Uric acid

*At termination, total protein measured in additional animals only.

At termination only on additional animals:

GGT
Total protein
Protein electrophoresis
Total bilirubin
LDH
CPK
Calcium
Sodium
Potassium
Chloride

The following urinalysis parameters, or as many as available sample would permit, were measured:

Appearance
Ames Multistix (pH, glucose, ketones, protein, bilirubin, blood, urobilinogen)
Volume
Specific gravity

Necropsies

All animals placed on test at study initiation, regardless of fate, were subjected to gross post mortem examination (necropsy) and tissue collection.

The necropsy included an examination of the external surface, all orifices, the cranial cavity, external and cut surfaces of the brain, the nasal cavity and paranasal sinuses, the spinal cord, the abdominal, thoracic, and pelvic cavities and their viscera, and the carcass. All gross findings were recorded.

The following tissues were preserved in AFA (alcohol-formalin-acetic acid) fixative:

Adrenal glands
Aorta
Bone marrow section (sternum and femur)
Brain (cerebrum, cerebellum, and pons)
Cecum
Colon
Esophagus
Eyes and contiguous Harderian glands
Gall bladder
Gonads
Heart
Kidneys
Liver (at least two lobes)
Lungs (two coronal sections including all lobes and main stem bronchii)
Lymph nodes (cervical and mesenteric)
Mammary gland
Muscle (skeletal)
Optic nerves
Pancreas
Parathyroid glands
Pituitary gland (fixed in situ)
Prostate
Salivary glands (submaxillary)
Sciatic nerve
Skin
Small intestine (duodenum, jejunum, and ileum)
Spinal cord (two levels)
Spleen
Stomach (cardiac, fundus, pylorus)
Thymus
Thyroid glands
Trachea
Urinary bladder
Uterus

In addition, all gross lesions and tissue masses were preserved. The entire head was preserved after the eyes and brain had been removed. Prior to terminal sacrifice, the pituitary was preserved in the head; at terminal sacrifice, it was removed and weighed.

Organ Weights

From animals sacrificed after clinical laboratory studies at 12 and 18 months, in addition to the terminal body weight, the following organs were weighed prior to fixation:

Heart
Liver
Spleen
Kidneys
Gonads
Brain

From animals sacrificed at termination (24 months), in addition to those listed above, the following organs were weighed prior to fixation:

Adrenals
Lung
Pituitary
Prostate
Salivary glands (sublingual)
Seminal vesicles
Thymus (or thymic remnant)
Thyroids
Uterus

Terminal organ weight to body weight and organ weight to brain weight ratios were computed.

Histopathology

Microscopic examination of tissues, lesions, and tissue masses was conducted on all animals placed on test at initiation, regardless of fate. In addition, from 10 animals per group selected at random at terminal sacrifice, two sections of spinal cord and three coronal sections through the head were examined.

Randomization

Animal assignment to test groups, original selection of animals for feed consumption data collection, selection of animals for feed consumption data collection after a death, and selection of animals for coronal head sections, were done using a random numbers table. Computer-generated randomizations were used for selection of animals for clinical laboratory studies, order of necropsies, and selection of diets for assay from Weeks 5-104.

Statistical Analysis

Body weight and feed consumption data, clinical pathology data, and terminal organ weight data were analyzed using analysis of variance and, when significant F ratios found, followed by Dunnett's t-test to determine significant differences between control and other treatment means. Incidences of pathologic lesions, where indicated, were analyzed using Chi square techniques. Survival data were analyzed using Cox's test for linear trends. Means which were statistically different at significance levels of 0.05 and 0.01 are indicated on the summary tables.

Retention of Records

All raw data including data books, individual pathology sheets, microscope slides, tissue blocks, a copy of the final report and a sample of the test material are the property of CIBA-GEIGY Corporation, but will be held in the archives of Hazleton Raltech, Inc., 3301 Kinsman Boulevard, Madison, Wisconsin.

RESULTS

Verification of Dose Levels

Results of diet assays for metolachlor concentration are presented in Tables 1 and 2. Assays of diets in the homogeneity study indicate that the procedures used for both the ethanol and "dry" mixed diets resulted in relatively homogeneous finished diets. The stability study data showed that metolachlor was stable in the finished diet for at least 4 weeks following mixing when held at room temperature. Time-weighted averages of assay results of diets from Week 1 through Week 105 indicate that animals were fed close to theoretical dose levels of 300, 1000, and 3000 ppm metolachlor in the diet.

Appendix 2. STATISTICS

005504 19
DATE: JULY 1, 1984

TITLE: METO.-FEMALES
REMARKS: I.S. AT 18 MO.

005504

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	8	2	2.5
300	8	1	2.5
1000	8	4	2.5
3000	8	3	2.5

NSUM= 32 OSUM= 10 ESUM= 10 BSUM= 10750 CSUM= 2.5225E+0
T= 2550 V= 9.70042E+06 Q= 1.36688E+07 SD= 3114.55 Z= .818739

p= .2065 79.35% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: I.S. AT 18 MO.

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	8	2	2.33333
300	8	1	2.33333
1000	8	4	2.33333

NSUM= 24 OSUM= 7 ESUM= 7 BSUM= 3033.33 CSUM= 2.54333E+
T= 1266.67 V= 908309 Q= 1.22889E+06 SD= 953.053 Z= 1.32906

p= .0919 90.81% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: I.S. AT 18 MO.

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	8	2	1.5
300	8	1	1.5

NSUM= 16 OSUM= 3 ESUM= 3 BSUM= 450 CSUM= 135000
T=-150 V= 58500.1 Q= 67500.1 SD= 241.868 Z=-.620173

p= .7324 26.76% PROBABILITY THAT THE EFFECT IS DOSE RELATED

DATE: JULY 17,198

TITLE: METOLACHLOR-FEMALES
REMARKS: TOT. ALV. CARCINOMA = DOT+IS+MS=TS

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	68	15	16.5
300	68	15	16.5
1000	68	17	16.5
3000	68	19	16.5

NSUM= 272 OSUM= 66 ESUM= 66 BSUM= 70950 CSUM= 1.66485E+
T= 7550 V= 6.85758E+07 Q= 9.02138E+07 SD= 8281.05 Z= .911721

P= .181 81.9% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: TOT. ALV. CARCINOMA = DOT+IS+MS=TS

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	68	15	15.6667
300	68	15	15.6667
1000	68	17	15.6667

NSUM= 204 OSUM= 47 ESUM= 47 BSUM= 20366.7 CSUM= 1.70767E+
T= 1133.33 V= 6.38139E+06 Q= 8.2511E+06 SD= 2526.14 Z= .448642

P= .3268 67.32% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: TOT. ALV. CARCINOMA = DOT+IS+MS=TS

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	68	15	15
300	68	15	15

NSUM= 136 OSUM= 30 ESUM= 30 BSUM= 4500 CSUM= 1.35E+06
T= 0 V= 530000 Q= 675000 SD= 728.011 Z= 0

P= .5 50% PROBABILITY THAT THE EFFECT IS DOSE RELATED

DATE: JULY 11, 1984

TITLE: METO.-MALES
REMARKS: I.S. AT 18 MO

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	8	0	2.75
300	8	4	2.75
1000	8	2	2.75
3000	8	5	2.75

NSUM= 32 OSUM= 11 ESUM= 11 BSUM= 11825 CSUM= 2.77475E+
T= 6375 V= 1.01854E+07 Q= 1.50357E+07 SD= 3191.46 Z= 1.99752

p= .0229 97.71% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: I.S. AT 18 MO

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	8	0	2
300	8	4	2
1000	8	2	2

NSUM= 24 OSUM= 6 ESUM= 6 BSUM= 2600 CSUM= 2.18E+06
T= 600 V= 824348 Q= 1.05333E+06 SD= 907.936 Z= .66084

p= .2544 74.56% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: I.S. AT 18 MO

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	8	0	2
300	8	4	2

NSUM= 16 OSUM= 4 ESUM= 4 BSUM= 600 CSUM= 180000
T= 600 V= 72000 Q= 90000 SD= 268.328 Z= 2.23607

p= .0127 98.73% PROBABILITY THAT THE EFFECT IS DOSE RELATED

NAME: LACAYO
TITLE: METOLACHLOR-MALES
REMARKS: TOTAL=LUT+MS=+IS+TS=ALL ALV. CARCINOMA

DATE: JULY 10,1984

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	68	11	14.9451
300	68	22	14.9451
1000	68	8	14.9451
3000	69	19	15.1648

NSUM= 273 OSUM= 60 ESUM= 60 BSUM= 64923.1 CSUM= 1.52774E+08
T= 6676.92 V= 6.46233E+07 Q= 8.25236E+07 SD= 8038.86 Z= .83058

P= .2031 79.69% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: TOTAL=DUT+MS=+IS+TS=ALL ALV. CARCINOMA

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	68	11	13.6667
300	68	22	13.6667
1000	68	8	13.6667

NSUM= 204 OSUM= 41 ESUM= 41 BSUM= 17766.7 CSUM= 1.48967E+07
T=-3166.67 V= 5.7795E+06 Q= 7.19778E+06 SD= 2404.06 Z=-1.31722

P= .9061 9.39% PROBABILITY THAT THE EFFECT IS DOSE RELATED

REMARKS: TOTAL=DUT+MS=+IS+TS=ALL ALV. CARCINOMA

DOSE LEVEL	No. of Animals	OBS. FREQ.	EXP. FREQ
0	68	11	16.5
300	68	22	16.5

NSUM= 136 OSUM= 33 ESUM= 33 BSUM= 4950 CSUM= 1.485E+06
T= 1650 V= 566501 Q= 742501 SD= 752.662 Z= 2.19222

P= .0142 98.58% PROBABILITY THAT THE EFFECT IS DOSE RELATED

005504

005504

003885

Metolachlor

Input Program

ct fisher def

```
1
  1.    //LHEX2CL JOB (WMJ1,352,A),LACAYO
  2.    /*CNTL NEG1DZT,SHR
  3.    // EXEC FORVLKGO,LIBDISK=FILE02,
  4.    // LIBNAME='NEG1DZT.STAT.LOAD'
  5.    //LOAD.SYSLIN DD *
  6.      INCLUDE SYSLIB(C2X2)
  7.      ENTRY MAIN
  8.    //GO.FT01F001 DD *
  9.    1 -1 1
 10.    .025 .025
 11.    -1
 12.    METOLACHLOR-MALES,CONTROL VS ALL DOSES AT 18 MO
 13.    0,8 11,24
 14.    -1
 15.    METO.-MALES,CONTROL VS ALL DOSES AT 24 MO.
 16.    5,20 26,84
 17.    -1
 18.    METO.-FEM,CONTROL VS ALL DOSES AT 18 MO
 19.    2,8 8,24
 20.    //
```

BEST AVAILABLE COPY

63

Meta-Fem Cntrl vs all doses at 24 mo.

DIAGNOSTIC MESSAGE DIRE

IEW0201 WARNING - OVERLAY STRUCTURE CONTAINS ONLY ONE SEGMENT -- OPTION CANCELED.

IFY215I VCVTH - ILLEGAL DECIMAL CHARACTER (0)
****** START OF BUFFER CONTENTS ******
0,8 11,24
****** END OF BUFFER CONTENTS ******
TRACEBACK OF CALLING ROUTINES; MODULE ENTRY ADDRESS=00145DA8
IFYVCVTH(0015A978) CALLED BY VLDIO## (00153168) AT ISN ## OFFSET).
NO ARGUMENTS PASSED TO SUBROUTINE
VLDIO## (00153168) CALLED BY MAIN (00145DA8) AT ISN ## OFFSET).
NO ARGUMENTS PASSED TO SUBROUTINE
MAIN (00145DA8) CALLED BY (OP/SYS)
0 STANDARD CORRECTIVE ACTION TAKEN, EXECUTION CONTINUING.
METOLACHLOR-MALES,CONTROL VS ALL DOSES AT 18 MO

TABLE(S):

0	8
11	13

TABLE(S) REORIENTED TO PREVENT ALL ODDS RATIOS BEING INFINITE

TABLE(S):

11	13
0	8

ODDS RATIO(S):

0.0000

ASYMPTOTIC MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 0.0000

ASYMPTOTIC TEST FOR MAIN EFFECT, P=0.2849E-01

*** WARNING, LOWER LIMIT MUST BE 0;
PROBABILITY REQUESTED FOR LOWER LIMIT INCLUDED IN UPPER LIMIT **

95.0% LIMIT PSI < 0.744307

CONDITIONAL MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 0.0000

EXACT TEST FOR MAIN EFFECT, P=0.1935E-01

EXACT CONFIDENCE LIMITS FOR PSI

95.0% LIMIT PSI < 9.6329 005504

*** SINGLE/COMBINED 2X2 TABLE PROGRAM JAN/16/84 *** CPU TIME=
SEC.
1 003885

METO.-MALES, CONTROL VS ALL DOSES AT 24 MO.

TABLE(S):

5 15
26 58

ODDS RATIO(S):

1.3448

ASYMPTOTIC MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 1.3448

ASYMPTOTIC TEST FOR MAIN EFFECT, P=0.4014

95.0% LIMITS 0.398443 < PSI < 4.771767

CONDITIONAL MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 1.3411

EXACT TEST FOR MAIN EFFECT, P=0.4097

EXACT CONFIDENCE LIMITS FOR PSI

95.0% LIMITS 0.406236 < PSI < 5.225135

*** SINGLE/COMBINED 2X2 TABLE PROGRAM JAN/16/84 *** CPU TIME=
SEC.
1

METO.-FEM, CONTROL VS ALL DOSES AT 18 MO

TABLE(S):

2 6
8 16

ODDS RATIO(S):

1.5000

ASYMPTOTIC MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 1.5000

ASYMPTOTIC TEST FOR MAIN EFFECT, P=0.5000

95.0% LIMITS 0.192134 < PSI < 13.827908

CONDITIONAL MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 1.4818

EXACT TEST FOR MAIN EFFECT, P=0.5118

*** SINGLE/COMBINED 2X2 TABLE PROGRAM JAN/16/84 *** CPU TIME= 0.000 SEC.

005504

METO.-FEM, CONTROL VS ALL DOSES AT 24 MO

003685

TABLE(S):

6 22
22 39

ODDS RATIO(S):

2.0684

ASYMPTOTIC MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 2.0684

ASYMPTOTIC TEST FOR MAIN EFFECT, P=0.1295

95.0% LIMITS 0.660099 < PSI < 6.725539

CONDITIONAL MAXIMUM LIKELIHOOD ESTIMATE OF PSI= 2.0523

EXACT TEST FOR MAIN EFFECT, P=0.1274

EXACT CONFIDENCE LIMITS FOR PSI

95.0% LIMITS 0.671159 < PSI < 7.144249

*** SINGLE/COMBINED 2X2 TABLE PROGRAM JAN/16/84 *** CPU TIME= 0.000 SEC.

0 MESSAGE SUMMARY: MESSAGE NUMBER - COUNT
0 215 1

005504
004725

Data Evaluation Record

Study Type: Primary DNA damage/repair assay.

Study Identification: "Autoradiographic DNA Repair Test on Rat Hepatocytes."

Lab. performing study:	Ciba-Geigy Limited Experimental Pathology Basle, Switzerland
Sponsor:	Ciba-Geigy Limited Agricultural Division Basle, Switzerland
Study no.:	831497
Accession no.:	258390
Report date:	November, 1984
Submitted to EPA:	6/14/85
Study authors:	Puri, E. and Muller, D.
Reviewed By:	D. Stephen Saunders Jr., Ph.D. Toxicologist, Section V TOX/HED (TS-769)
Approved By:	Irving Mauer, Ph.D. Geneticist, Toxicology Branch Hazard Evaluation Division (TS-769);

10-11-85

Conclusions: No effect of treatment on the mean number of silver grains per nucleus was apparent at any of the tested concentrations of metolachlor. The positive control induced the appropriate response, demonstrating that the test system was sensitive to a known mutagen.

The study is deficient because range-finding data were not submitted to support the selection of test article concentrations, nor was any evidence of cytotoxicity presented in the main study. Current guidelines for in vitro mutagenicity studies require that chemicals be tested to the limits of cytotoxicity or solubility.

Classification: Unacceptable Deficiencies as noted.

Materials and Methods

A. Materials- (1) Test chemical: Metolachlor CGA 24 705 technical, batch no. op. 303010, 95.9% a.i.
Positive control: dimethylnitrosamine (DMN).

(2) Concentrations tested: 0.25, 1.25, 6.25 or 31.25 nl/ml of metolachlor; DMN- 100 mM.

(3) Test species: Primary rat hepatocytes isolated from a single adult male rat (Tif: RAIf[SPF]) obtained from Ciba-Geigy Tierfarm, Sisseln.

Materials and Methods (con't)

B. Methods: A photocopy of the submitted methods is appended. The methods were reviewed and the following point was noted:

1) Although the methods stated that doses were selected on the basis of a range-finding assay, these data were not submitted.

Results/Discussion

Data were submitted as summary data and as individual findings.

No effect of treatment on the mean number of silver grains/nucleus, an index of DNA repair due to incorporation of ^{3}H-thymidine, was apparent (Table 1, photocopied from the study report). The positive control, DMN, induced a large increase in this value, demonstrating that the test system could respond to a known mutagen.

The study is deficient because data were not submitted to demonstrate the doses at which metolachlor was cytotoxic to target cells. Therefore, it cannot be determined whether the doses of test material were sufficient.

Classification: Unacceptable Range-finding data not submitted, no evidence of cytotoxicity in the main study.

Table 1

AUTORADIOGRAPHIC DNA REPAIR TEST ON RAT HEPATOCYTES

No. of experiment: 831497 Test substance: CGA 24 705 techn., Batch No.: op. 303010

Treatment groups	Concentration	Silver grains/nucleus ($\bar{x}$)
Negative control: culture medium	---	0.99
Negative control: vehicle	---	1.53
Positive control: DMN	100 mM	15.8
Test substance, highest concentration	31.25 nl/ml	1.37
Test substance, 1/5 of highest concentration	6.25 nl/ml	1.58
Test substance, 1/25 of highest concentration	1.25 nl/ml	1.62
Test substance, 1/125 of highest concentration	0.25 nl/ml	1.67

PROCEDURE

A toxicity test was first performed to determine the highest concentration to be used in the DNA-repair assay. The concentration best suited as the highest to be used in the DNA-repair test is determined by reference to three criteria: 1. a sufficiently large number of cells must adhere to the cover-slips; 2. at least 25% of the cells must show viability upon examination by means of the vital-staining technique; 3. a corresponding percentage of the cells must be in good condition upon morphological examination.

Freshly isolated hepatocytes from a male rat (Tif.RAIf(SPF), weight: 178 g) were cultivated in WILLIAMS' Medium E containing 10% foetal bovine serum. A series of compartments in Multiplates containing gelatinized THERMANOX cover-slips was seeded with 4×10^5 cells per compartment (density 10^5 cells/ml; 4 ml/compartment). The cells were allowed to attach to the cover-slips during an attachment period of 1.5-2 hours. They were then washed and cultivated overnight in renewed medium (adhesion period). The compartments were filled with 4 ml of culture medium during the attachment period and with 2 ml during the adhesion period.

On the following morning, the test substance was dissolved in DMSO and seven stock solutions were prepared. From each, a volume of 20 µl was added to two compartments containing 2 ml medium. The highest of the seven final concentrations was 500 nl/ml, the lowest 7.81 nl/ml. In addition, a negative control containing the vehicle only was run.

After an incubation period of five hours the medium was removed and the cells were washed twice with BSS and stained with Trypan-blue solution (0.2%) for five minutes. After washing with BSS, the cells were fixed and the percentage of unstained cells evaluated by counting 100 cells.

The DNA-repair assay was likewise performed with cells freshly isolated from a male rat (Tif.RAIf(SPF), weight: 212 g). The procedure employed during the attachment period and the adhesion period was the same as described in the previous toxicity test. At the end of the adhesion period, compartments were then treated under each of the following conditions: four preselected concentrations of the test substance; a positive control (dimethylnitrosamine, DMN, MERCK, 100 mM) a negative control containing the vehicle (DMSO) and an untreated negative control.

From the results obtained in the toxicity test, the highest usable concentration was calculated to be 31.25 nl/ml. Three further, lower concentrations were calculated, diminishing by a factor of 0.2. From the test substance and from the positive control substance stock solutions were prepared, from each of which 20 µl was added to the volume of 2 ml in the compartment. In the case of negative controls corresponding volumes of the vehicle and of culture medium were added.

Immediately after addition of the test substance, ^{3}H-thymidine was added (6-^{3}H-thymidine, specific activity 24.6 Curies/mmol, THE RADIOCHEMICAL CENTRE, Amersham, England, Batch: 125). 8 µCi in 8 µl was added to 2 ml medium in the compartment. At the end of the incubation period of 5 hours the cells were washed twice with BSS and fixed with ethanol/acetic acid, 3/1, v/v. The cover-slips were mounted on microscope slides and prepared for autoradiography. The exposure time was 6 days. The autoradiographs were stained with haematoxylin-eosin.

The background in the autoradiographs was determined in cell-free areas microscopically. It was found to be negligibly low. From each of the treatment groups and from the positive and the negative controls 150 nuclei in altogether three slides (50 cells/slide) were scored. Counting of silver grains over the nuclei of the

Data Evaluation Record

Study Type: Primary DNA damage/repair assay.

Study Identification: "Autoradiographic DNA Repair Test on Human Fibroblasts."

Lab. performing study: Ciba-Geigy Limited
Experimental Pathology
Basle, Switzerland

Sponsor: Ciba-Geigy Limited
Agricultural Division
Basle, Switzerland

Study no.: 831499
Accession no.: 258390
Report date: November, 1984
Submitted to EPA: 6/14/85
Study authors: Puri, E. and Muller, D.

Reviewed By: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Approved By: Irving Mauer, Ph.D.
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

Conclusions: No effect of treatment on the mean number of silver grains per nucleus was apparent at any of the tested concentrations. The positive control induced the appropriate response, demonstrating that the test system was sensitive to a known mutagen.

The study is deficient because range-finding data were not submitted to support the selection of test article concentrations, nor was any evidence of cytotoxicity presented in the main study. Current guidelines for in vitro mutagenicity studies require that chemicals be tested to the limits of cytotoxicity or solubility. Also, the effect of metabolic activation was not assessed.

Classification: Unacceptable Deficiencies as noted.

Materials and Methods

A. Materials- (1) Test chemical: Metolachlor CGA 24 705 technical, batch no. Op. 303010, 95.9% a.i.
Positive control: 4-nitroquinoline-N-oxide (4NQO).

(2) Concentrations tested: 0.125, 0.625, 3.125 or 15.625 ml/ml of metolachlor; 4NQO- 5 uM.

(3) Test species: Human fibroblasts (CRL 1121), obtained from ATCC, Rockville, MD.

Materials and Methods (con't)

B. Methods: A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

1) Although the methods stated that doses were selected on the basis of a range-finding assay, these data were not submitted.

2) The effect of metabolic activation was not assessed.

Results/Discussion

Data were submitted as individual values with calculated means and variances, and are summarized in Table 1 (photocopied from the study report).

No effect of treatment on the mean number of silver grains per nucleus was noted. Since no S-9 incubations were conducted, the effect of metabolic activation was not assessed. The positive control, 4NQO, produced a large increase in the number of silver grains/nucleus, demonstrating that the test system could respond to a known mutagen.

Classification: Unacceptable Range-finding data not submitted, no assessment of metabolic activation, no evidence that metolachlor was tested to the limits of cytotoxicity.

Table 1

AUTORADIOGRAPHIC DNA REPAIR TEST ON HUMAN FIBROBLASTS

No. of experiment: 831499 Test substance: CGA 24 705 techn. Batch No.: op. 303010

Treatment groups	Concentration	Silver grains/nucleus ($\bar{x}$)
Negative control: culture medium	---	1.11
Negative control: vehicle	---	0.91
Positive control: 4NQO	5 µM	38.7
Test substance, highest concentrations	15.625 nl/ml	0.75
Test substance, 1/5 of highest concentration	3.125 nl/ml	0.85
Test substance, 1/25 of highest concentration	0.625 nl/ml	1.07
Test substance, 1/125 of highest concentration	0.125 nl/ml	1.01

PROCEDURE

A toxicity test was first performed to determine the highest concentration to be used in the DNA-repair assay. The concentration best suited as the highest to be used in the DNA-repair test is determined by reference to three criteria: 1. a sufficiently large number of cells must adhere to the cover-slips; 2. at least 25% of the cells must show viability upon examination by means of the vital-staining technique; 3. a corresponding percentage of the cells must be in good condition upon morphological examination.

Human fibroblasts (CRL 1121 from "The American Type Culture Collection, Rockville, Md, U.S.A.") were cultivated in Dulbecco's Minimal Essential Medium containing 10% foetal bovine serum. A series of compartments in Multiplates containing glass cover-slips was seeded with 3×10^4 cells per compartment (1 ml medium/compartment) and cultivated overnight. On the following morning, the test substance was dissolved in DMSO and seven stock solutions were prepared. From each, a volume of 10 µl was added to two compartments containing 1 ml medium. The highest of the seven final concentrations was 500 nl/ml, the lowest 7.8125 nl/ml. In addition, a negative control containing the vehicle only was run.

After an incubation period of five hours the medium was removed and the cells were washed twice with BSS and stained with Trypan-blue solution (0.2%) for five minutes. After washing with BSS, the cells were fixed and the percentage of unstained cells evaluated by counting 100 cells.

The DNA-repair assay was likewise performed. The procedure employed for the preparation of 35 compartments was the same as described in the previous toxicity test.

hepatocytes was carried out with the aid of an electronic counter (ARTEK Model 982) attached to a microscope (ZEISS) at a magnification of 2000x, using an objective 100x and a projective 10x.

Cells which were in the DNA-synthesis phase showed more than 120 silver grains/nucleus. The percentage of such cells was about 0.2. These cells were excluded from the determination of the silver grain/nucleus count.

The test substance is generally considered to be mutagenic or carcinogenic if the mean number of silver grains per nucleus in relation to the negative controls is more than doubled at any concentration.

The compartments were treated under each of the following conditions: four preselected concentrations of the test substance; a positive control (4-nitroquinoline-N-oxide, 4NQO, FLUKA, 5 µM); a negative control containing the vehicle (DMSO) and an untreated negative control.

From the results obtained in the toxicity test, the highest usable concentration was calculated to be 15.625 nl/ml. Three further, lower concentrations were calculated, diminishing by a factor of 0.2. From the test substance and from the positive control substance stock solutions were prepared, from each of which 10 µl was added to the volume of 1 ml in the compartment. In the case of negative controls corresponding volumes of the vehicle and of culture medium were added.

Immediately after addition of the test substance, ^{3}H-thymidine was added (6-^{3}H-thymidine, specific activity 24.6 Curies/mmol, THE RADIOCHEMICAL CENTRE, Amersham, England, Batch: 125). 2 µCi in 4 µl was added to 1 ml medium in the compartment. At the end of the incubation period of 5 hours the cells were washed twice with BSS and fixed with ethanol/acetic acid, 3/1, v/v. The cover-slips were mounted on microscope slides and prepared for autoradiography. The exposure time was 6 days. The autoradiographs were stained with haematoxylin-eosin.

The background in the autoradiographs was determined in cell-free areas and found to be negligibly low. From each of the treatment groups and from the positive and the negative controls 200 nuclei in altogether four slides (50 cells/slide) were scored, the number of silver grains counted, and the mean values calculated.

Cells which were in the DNA-synthesis phase showed more than 120 silver grains/nucleus. These were excluded from the determination of the silver grain/nucleus count.

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Data Evaluation Record

Study Type: Micronucleus test in Chinese hamsters.

Study Identification: "Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster."

Lab. performing study: Ciba-Geigy Limited
Experimental Pathology
Basle, Switzerland

Sponsor: Ciba-Geigy Limited
Agricultural Division
Basle, Switzerland

Study no.: 831498
Accession no.: 258390
Report date: October, 1984
Submitted to EPA: 6/14/85
Study authors: Strasser, F. and Arni, P.

Reviewed By: D. Stephen Saunders Jr., Ph.D. 10-11-85
Toxicologist, Section V
TOX/HED (TS-769)

Approved By: Irving Mauer, Ph.D. 10-11-85
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

Conclusions: No effect of treatment on the incidence of induction of micronuclei in Chinese hamsters was apparent. The positive control induced the appropriate response, demonstrating that the test system was sensitive to a known mutagen.

The study is deficient because data were not submitted to demonstrate that the test article reached the target tissue, the bone marrow.

Classification: Inconclusive Deficiencies as noted.

Materials and Methods

A. Materials- (1) Test chemical: Metolachlor CGA 24 705 technical, batch no. op. 303010, 95.9% a.i.; Positive control: cyclophosphamide.

(2) Doses tested: Single doses of 0, 1250, 2500 or 5000 mg/kg of metolachlor by gavage; cyclophosphamide 128 mg/kg in distilled water, method of administration not specified.

(3) Test animal: Male and female random outbred Chinese hamsters, obtained from Ciba-Geigy Tierfarm, Sisseln.

Materials and Methods (con't)

B. Methods: A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

1) Although the methods stated that doses were selected on the basis of a range-finding study, these data were not submitted.

2) The route of administration of the positive control, cyclophosphamide, was not specified.

Results/Discussion

No effect of treatment on the incidence of induction of micronuclei was apparent (Table 1, photocopied from the study report). The positive control, cyclophosphamide, caused an increase in the induction of micronuclei, demonstrating that the test system was sensitive to the effects of a known mutagen.

Although doses were reportedly selected on the basis of a range-finding study, these data were not submitted. More importantly, no data were presented to demonstrate that the test article was absorbed from the gut and reached the target tissue, the bone marrow, in sufficient concentration to produce a mutagenic effect.

Classification: Inconclusive No evidence that the test article reached the target tissue.

Table 1

No. of experiment: 830[illegible]

THE EFFECT OF CGA 24 705 techn. AND CYCLOPHOSPHAMIDE ON BONE MARROW CELLS OF CHINESE HAMSTER

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Animals sacrificed 24 h after the second application

	Number of animals	Sex of animals	Percent of cells with anomalies of nuclei: Single Jolly bodies	Fragments of nuclei in erythrocytes	Micronuclei in erythroblasts	Micronuclei in leucopoietic cells	Polyploid cells	Total
Control (dist.water)	1	♀	0.1	0.1				0.2
	2	♀						0.0
	3	♀						0.0
	4	♂						0.0
	5	♂						0.0
	6	♂						0.0
Cyclophosphamide (128 mg/kg)	1	♀	8.9	2.6	0.9	0.1	0.2	12.7
	2	♀	13.2	2.6	0.9	.	0.1	16.8
	3	♀	8.8	1.7	1.9	0.2		12.6
	4	♂	7.4	1.7	1.0	0.1		10.2
	5	♂	5.3	1.8	0.8	0.2	0.1	8.2
	6	♂	11.7	2.9	1.5	0.1	0.1	16.3
CGA 24 705 techn. (1250 mg/kg)	1	♀	0.3					0.3
	2	♀	0.1					0.1
	3	♀	0.2					0.2
	4	♂						0.0
	5	♂						0.0
	6	♂	0.1					0.1
CGA 24 705 techn. (2500 mg/kg)	1	♀	0.2					0.2
	2	♀	0.1					0.1
	3	♀						0.0
	4	♂						0.0
	5	♂						0.0
	6	♂						0.0
CGA 24 705 techn. (5000 mg/kg)	1	♀	0.2					0.2
	2	♀	0.1					0.1
	3	♀	0.2					0.2
	4	♂	0.1					0.1
	5	♂						0.0
	6	♂						0.0

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PROCEDURE

1. Data on the animals used

Chinese hamsters (Cricetulus griseus) of either sex (♀:♂ = 1:1) (weight ♀♀ 22-27 g, ♂♂ 24-31 g; age ♀♀ 6-10 weeks, ♂♂ 4-9 weeks) were used. Standard diet: NAFAG No.924. Tap water ad libitum. The animals were kept in an air-conditioned room at a temperature of 23-24°C and a relative humidity of 56-65%. The room was illuminated for 12 hours daily. Identification of the animals by individual caging.

2. Tolerability test

A preliminary test was performed to determine the highest dosage of the test substance to be applied in the mutagenicity assay.

The tolerability test is carried out step by step. In the first instance four Chinese hamsters (2 ♂♂ and 2 ♀♀) are treated with the dose corresponding to the LD_{50} value. Treatment consists of one daily dose on each of two consecutive days. The observation period corresponds to the interval between first administration and sacrifice of the animals in the mutagenicity test. If all animals die in the first step, a second test is performed in which the highest dose given is 1/3 of the applied dose used in the preceding test. If some of the animals die, the test is continued with a high dose corresponding to 1/2 of that dose. Depending on the outcome the highest dose causing no deaths is used as the highest in the mutagenicity test, or if necessary the test is repeated with lower doses.

The oral LD_{50} was found to be >5000 mg/kg in Chinese hamsters of either sex (cf. Lab.Report: GU 2, dated February 3, 1984).

In this preliminary test the dose of 5000 mg/kg was determined as the highest applicable in the mutagenicity assay, together with further two doses, diminishing by a factor of 0.5.

3. Treatment schedule in the mutagenicity test

The preparation was administered orally to groups of 6 female and 6 male animals each. Treatment consisted of daily one application on 2 consecutive days. 24 h after the second application the animals were sacrificed by dislocation of the cervical vertebrae.

a) CGA 24 705 techn.: 1250, 2500 and 5000 mg/kg in 20 ml/kg distilled water.

b) Cyclophosphamide (ENDOXAN®): 128 mg/kg in 20 ml/kg dist. water (positive control).
Manufacturer of ENDOXAN®: ASTA-Werke, Germany.

c) 20 ml/kg dist. water (negative control).

4. Preparation of bone marrow

Bone marrow was harvested from the shafts of both femurs. In a siliconized pipette filled with approx. 0.5 µl rat serum the bone marrow was drawn up. In order to receive a homogeneous suspension the content of pipette was aspirated gently about three times. Small drops of the mixture were transferred on the end of a slide, spread out by pulling it behind a polished cover glass and the preparations were air-dried. Three hours later, the slides were stained in undiluted May-Grünwald solution for 2 min then in May-Grünwald solution/water 1/1 for 2 min and then in Giemsa's, 40% for 20 min. After being rinsed in methanol 55% for 5-8 sec and washed off twice in water, they were left immersed in water for approx. 2 min. After rinsing with distilled water and air-drying, the slides were cleared in Xylene and mounted in Eukitt.

5. Scoring of the slides

The slides of three female and three male animals each of the negative control group, the positive control group and of the groups treated with various doses of CGA 24 705 techn. were examined. 1000 bone marrow cells each were scored per animal and the following anomalies were registered:
a) Single Jolly bodies, b) fragments of nuclei in erythrocytes, c) micronuclei in erythroblasts, d) micronuclei in leucopoietic cells, e) polyploid cells.

6. Statistics

The significance of difference was assessed by χ^2-test.

Reviewed by Laurence D. Chitlik
Toxicologist
Toxicology Branch HED

(s) Laurence D. Chtlik
M. Adrian Gross

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DATA REVIEW:

Dog, 6-Month Oral Toxicity Study, CGA-24705 Technical, International Research and Development Corporation, Report#382-054, November 2, 1979

Twenty-eight male and 28 female beagle dogs 4 to 6 months of age were used in this study. Dogs were housed individually in wire-mesh cages and conditioned in the laboratory for 9 weeks prior to initiation of the study. They were also vaccinated for hepatitis, distemper, leptospirosis, treated for intestinal worms, checked for heart worms and given an ophthalmologic examination. Blood and urine samples were also taken during this period and unhealthy dogs were eliminated from the study. Dogs were then randomized and assigned to the following groups:

Test Level (ppm)	Number of Dogs	
	Male	Female
0 (control)	8	8
100	6	6
300	6	6
1000	8	8

CGA-24705 Technical was dissolved in ethanol to prepare a 50% (w/v) solution used to make a premix and mixed with the remaining Purina Dog Chow.

Observations

Dogs were observed daily for appearance, behavior, and mortality. Tissue mass incidence, body weights and food consumption were determined weekly. Compound intake was calculated weekly.

During the pretest period and at 6 months an ophthalmoscopic examination was performed on each dog.

Clinical Tests

Hematology and blood chemistry tests were performed initially and at monthly intervals including the recovery period. Urinalysis was performed initially and at 2, 4, 6 months and during the recovery period. The following determinations were made:

Hematology

1. hemoglobin
2. hematocrit
3. erythrocyte count
4. total and differential leuckocyte count
5. platelet count
6. reticulocyte count (beginning at 4 months)
7. prothrombin time
8. activated partial-thromboplastin time (APTT)
9. methemoglobin
10. Heinz bodies

Blood Chemistry

1. BUN
2. fasted blood glucose
3. total cholesterol
4. total protein
5. serum calcium
6. serum potassium
7. serum sodium
8. serum chloride
9. direct and total bilirubin
10. SGOT
11. SAP
12. SGPT
13. LDH

Urinalysis

1. specific gravity
2. microscopic sediment
3. protein
4. glucose
5. ketones
6. bilirubin urobilinogen
7. pH
8. occult blood (only months 4, 6, 7)

Gross Pathology

Dogs were sacrificed by exsanguination after an over-dose of sodium pentobarbital and then necropsied. "Selected tissues" and liver, kidneys, heart, brain, spleen, gonads, adrenals, thyroids (with parathyroid) and the pituitary were weighed. Recovery animals (2/sex, group I and IV) were sacrificed in the same manner, 1 month after dosing was completed.

Histopathology

The following tissues were stained with hematoxylin and eosin and examined microscopically.

adrenal gland
aorta
bone marrow
brain (3 levels)
cecum
colon
esophagus
eye
gall bladder
gonads
heart
kidney
liver
lung with bronchi
lymph node (cervical and mesenteric)
mammary gland
muscle
thymus
optic nerve
pancreas
parathyroid
peripheral nerve (sciatic)
prostate
salivary gland (submaxillary)
skeletal muscle
skin
small intestine (3 levels)
spinal cord (2 levels)
spleen
stomach (3 levels)
thyroid with parathyroid

Statistical Evaluation

Statistical analysis included one-way analysis of variance, Bartlett's test for homogeneity of variances and the appropriate t-test for equal or unequal variances using Dunnett's multiple comparison tables to determine significance. The Wilcoxin, Mann, Whitney, Rank Sum test was also used in the 1/21/80 and 2/6/80 addendums.

Diet Analysis

Prior to the start of the study and at one week intervals 100g diet samples were sent to Ciba-Geigy for analysis. Also, 1 gram of the technical material was sent for analysis at 3 and 6 months.

RESULTS

Diet analysis revealed that the average low dose was 92 ppm, the intermediate group received 273 ppm and the high dose received 952 ppm. By time weighting these, the low dose received 88.6 ppm, the intermediate group 270 ppm and the high dose 964.8 ppm. The percentage error is - 4-11% and not significant.

Observations

Emesis, soft stool and ocular discharge, interdigital cysts, relaxed nictitating membrane and slight dermatitis was observed in all groups including controls at some point during the study.

A "thickened area" along the mammary cord was noted in two female controls, three of the 300 ppm females and five 1000 ppm females. Individual animal observations were not included in the report.

No deaths occurred during the study.

Mean body weight data demonstrated that 1000 ppm males and females gained less body weight than controls and other test groups.

Although food consumption was slightly reduced in 1000 ppm males (2% reduction) and females of the 100 ppm level (6% reduction), 300 ppm level (5% reduction) and 1000 ppm level (9% reduction), the only significant decrease in food consumption is demonstrated by the 1000 ppm females. The average compound consumption for male dogs was determined to be 2.92 (100 ppm level), 9.71 (300 ppm level), and 29.61 mg/kg/day and for female dogs it was determined to be 2.97 (100 ppm level), 8.77 (300 ppm level) and 29.42 mg/kg/day (1000 ppm level). After examinations of 4 randomly selected dogs for ophthalmologic examinations at 6 months, it was concluded that the increase in punctate corneal opacities (epithelial or subepithelial lesions) in all groups was suggestive of trauma and not compound related.

Hematology

Male dogs at the 300 and 1000 ppm levels demonstrated significantly reduced activated partial thromboplastin time (APTT) at 5 and 6 months ($p < 0.01$) as follows:

	Control (seconds)	100 PPM	300 PPM	1000 PPM
5 months	11.6	11.0	10.0*	10.0*
6 months	11.5	12.4	9.6*	9.7*

*designated in the report as significant

In female dogs APTT values were also statistically significantly less than controls at month 4 (100 ppm level), at month 6 at 300 ppm level, at month 5 for the 1000 ppm level and at the end of the recovery period in the 1000 ppm females. As noted below:

Month	Control (seconds)	100 PPM	300 PPM	1000 PPM
4	11.0	10.4*	11.8	10.9
5	10.9	11.0	11.0	9.8**
6	11.3	11.6	9.9*	10.3
7 (recovery)	11.0	-	-	9.3**

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

Considering that the APTT is reduced (rather than lengthened) in both males and females and to statistically significant levels, potential methodology error is considered. Sample preparation is the most likely source of error and the test must also be run under carefully controlled conditions.

These points were discussed with Dr. Darrel Summer of Ciba-Geigy on 1/9/80, and the precise procedure as well as explanations for these irregular values were requested.

Dr. Summer telephoned on 1/10/80, after consulting with IRDC personnel. He explained that IRDC agreed that they also had not observed shortened APTT values except in this study and that they were at a loss to explain it. The question of sample preparation was discussed as well as which test group was sampled first. Dr. Summer agreed to further research the methodology for the source of the problem and submit this in writing. He also agreed to submit copies of unrevised pages in the report (Note: A number of report pages are labeled as "revised" and he explained that most revisions were due to typing errors and that some additional explanation had been added to the report on the SAP findings).

Considering that Prothrombin times did not demonstrate significant effects, added credence was given to the theory that the APTT findings (since shortened) could be considered as erroneous due to faulty methodology. Dr. Summer agreed to submit the APTT methodology used, and make an effort to determine the source of the problem.

Since a number of APTT findings were significant of $p < 0.01$, and the effect appeared dose related, it was decided to pursue this issue further.

On 1/17/80, Ciba-Geigy submitted an addendum to their 6 month dog study in an attempt to answer questions related to their very irregular APTT values. The document included the following:

1. Dade instructions for the use of Actin (Activated Cephaloplastin Reagent) for APTT determinations.

2. An instruction manual for the MLA Electra Coagulation timers E620 and E650.

3. APTT values from 4 other study control groups.

4. Dade I and Dade II calibration control data.

5. A short letter from Dr. A. Clark Kahn III, Director of Clinical Pathology.

6. Copies of original report pages not submitted along with the report.

Unfortunately, the information provided (especially the letter from Dr. Kahn) did not seriously attempt to resolve the APTT methodology problems at IRDC. No information related to sample gathering (ie - redomization) nor sample preparation (the most likely source of error) were included. The only attempt at resolving the questions raised including the following statement by Dr. Kahn:

> "There is a possibility of interference from elevated temperature and interference by unknown particulates."

Other points brought out in the letter from Dr. Kahn which are of interest include:

1. Dr. Kahn believes that "this particular test cannot be interpreted clinically based on shortened reaction times."

2. He also indicated that APTT values are within the range based on other control values obtained from other studies.

A comparison of APTT data from this study to the four other control APTT groups, demonstrated that a number of test group values were above and below this range.

Dr. Barnett of Ciba-Geigy was informed by telephone on 1/17/80, that the addendum did not answer the questions posed concerning IRDC methodology, especially sample preparation. Dr. Barnett agreed that the submission was inadequate and indicated that another effort would be made.

On 1/21/80, Ciba-Geigy submitted a second addendum to this study (EPA Accession#099203) which also included discussion of the APTT problems. The following additional information relative to this issue was included in this submission:

1. Summary/discussion of the IRDC APTT method and associated problems (prepared by Ciba-Geigy).

   A new attempt was made to use data from 4 previous IRDC control groups to demonstrate that test group values were within the "normal range".

   Considering (1) the times were not monitored between sample collection and processing on the MLA-600 coagulation instrument, (2) that controls were sampled first and (3) no SOP was in existence before or during this study (Verified by discussion with Dr. John Barnett of Ciba-Geigy) (4) it must therefore be assumed that the "normal range" based upon 4 previous control groups (where APTT values were determined under the same conditions) is also of no value for comparison purposes. Furthermore, as demonstrated by one of these control groups, the standard deviation varied by more than 4x from one study to another.

Dr. Kahn's letter of 1/10/80, also indicated that a sex related difference may exist, yet the Ciba-Geigy manipulation of the data combined male and female data. This is rather conflicting and certainly not appropriate.

The use of this control data, compiled from four previous IRDC studies, to establish the normal range for APTT values is therefore not acceptable. Once correct and uniform procedures are established at IRDC, a normal range should be established excluding the submitted control data values. Furthermore, comparing the normal range proposed by Dr. Kahn to another that he referenced in his 1/21/80 letter, of another facility (Laboratory of Dr. Hugh Lewis, normal range of 9.3 - 11.6 seconds (based upon 3 SDV rule) with a mean of 10.3 seconds) many more of his test and control values would be outside this range since it is approximately half the time of Kahn's range.

2. Ciba-Geigy toxicologists determined after visting IRDC and going through the APTT procedure with Dr. Kahn that possible sources of error included:

   A. Optical interference induced by a technician pouring off supernatant and including some blood cells. (Not a likely

B. If insufficient sodium citrate were added, the clotting process could have been initiated prematurely. (Not considered likely problem).

C. The procedures used at IRDC did not limit the time between sample collection and analysis.

D. Ignoring the significance of point 3 above, IRDC claimed that reported values were within the lower limit of the normal range and that the statistical significance is a Type I error (false positive).

3. Also in reference to the APTT question, Ciba-Geigy submitted a second letter from Dr. Kahn dated 1/21/80. Dr. Kahn indicated neither the time of blood sampling of the dogs nor the time when they were processed was recorded. Dr. Kahn also responded to a telephone request for the APTT SOP that, "all SOP's are the sole property of IRDC and cannot be released." This statement is inconsistent with the Ciba-Geigy letter of Jack Norton, 1/28/80. In this letter Norton states that, "Ciba-Geigy was informed by IRDC that an SOP had not been instituted for APTT."

The Kahn letter also referenced a telephone conversation of 1/20/80, with this reviewer where we discussed the fact that APTT times for dogs at IRDC are several times shorter than human values and perhaps that IRDC was not abe to properly control processing time delays in the animal studies and obtain valid APTT normal range tolerances (ie - the IRDC range is 5.4 seconds while another laboratory referenced by Dr. Kahn, that of Dr. Hugh Lewis, had a range of 2.3 seconds).

The question was raised by this reviewer whether IRDC had considered <u>not</u> activating the thromboplastin times and whether the values would then be more reliable. Dr. Kahn didn't answer this question at the time, but his memo indicates that he contacted the "supervisor of coagulation testing" at the laboratory of Dr. Hugh Lewis. She indicated the method is routinely used by them, but as mentioned earlier, the APTT range at this facility is much tighey than at IRDC!

Dr. Kahn then inappropriately tried to compare the lower APTT values in the metolachlor study to the normal range at the Lewis laboratory. He also did not realize that in such a comparison, <u>many</u> values in this study would have then been outside the upper range limit. The wide disparity in ranges definitely indicates APTT methodology problems at IRDC.

Dr. Kahn indicated that some findings were unusually low in this study and time intervals between obtaining the sample and the completion of the APTT were "not rigidly controlled". He also issued a directive that all coagulation tests must henceforth be completed within four hours after obtaining the blood sample.

The letter of Jack Norton, 1/28/80, Ciba-Geigy, also addressed the wider APTT normal range at IRDC and concluded that some variable is not controlled at IRDC and that it is likely due to delays in analysis of samples. He also stated that the position of Ciba-Geigy is "the reduced APTT values reported in this study are not meaningful in regard to the toxicity of metolachlor."

This reviewer agrees with the registrant that APTT values in this study are not related to a compound effect and are due to incorrect methodology.

The platelet count was significantly increased in 300 ppm females during the pretest period (364 10^3/cmm) while decreased in the 1000 ppm females at the first month. An increase was also noted during the 4th month in 1000 ppm females ($p < 0.05$). It was also increased as compared to controls (the control value is relatively low at 161 10^3/cmm) in the 100 ppm females at 3 months (244 10^3/cmm) with $p < 0.01$.

Males at 300 ppm showed a significant decrease in platelets at 3 months (323 10^3/cmm as compared to controls 368 10^3./cmm). These fluctuating and inconsistent values are not considered compound related.

In male dogs of the 300 ppm level at 3, 4, and 5 months both the erythrocyte count and the hemoglobin concentration were significantly less than in control dogs. At the 1000 ppm level, during month 3, the erythrocyte count was also reduced in males. The hematocrit was also reduced in males during months 3 & 4. These values were within the normal range, with the slight exception of the low erythrocyte counts at 3 and 4 months in the 300 ppm males (5.71 and 5.54 X 10^6/cmm).

Considering the sporadic nature of these findings, that they did not persist to termination, and the variation in normal range, these findings are not considered compound related. There were no effects demonstrated in the females.

Serum alkaline phosphatase levels generally decreased more slowly in the test groups as compared to controls. At 4 months, the mean SAP level in 1000 ppm males was statistically significantly higher ($p < 0.05$) at 97 int'l u/L than the control level of 66 int'l u/L. At 6 months both the 300 ppm level males at 78 int'l u/L ($p < 0.01$) and the 1000 ppm males at 87 int'l u/L ($p < 0.05$) were significantly higher than the control SAP of 56 int'l u/L. At 100 ppm, males also had a higher SAP level of 77 but this did not show statistical significance. In females at 6 months the 1000 ppm level of 100 int'l u/L was statistically higher than controls at 69 int'l u/L. Both the 100 ppm level of 86 and the 300 ppm level of 83 int'l u/L were also higher than the mean control level at 6 months. After a one month recovery period the SAP level in two 1000 ppm females dropped to 53 int'l u/L indicating that this effect was compound related.

The lower rate of decrease of SAP as noted above may be associated with decreased bone maturation (possibly related to a retarded growth rate), or hepatic disease/dysfunction. Microscopic examination of liver and bone marrow revealed no unusual findings. On the other hand body weight gain, although not statistically significant was slightly reduced in both males and females of the 300 and 1000 ppm groups and males of the 100 ppm group after 6 months on test.

	Control	100 PPM	300 PPM	1000 PPM
Males	2.6 kg	1.6 kg	2.1 kg	1.3 kg
Females	2.1 kg	2.1 kg	1.9 kg	1.4 kg

Food consumption was also slightly reduced in 1000 ppm females which may have been related to effects in this group but it does not explain other findings.

It is therefore concluded that the slower rate of SAP decrease in 1000 ppm males and females and 300 ppm males in a toxic response to the test compound. This reviewer also notes that repeat determinations were carried out for SAP levels (only during month 6) in two males at 100, 300, and 1000 ppm levels and without explanation. Dr. John Barnett of Ciba-Geigy was telephoned on 1/15/80, and an explanation for the repeats was requested.

Dr. Barnett checked with IRDC and determined that the repeats were due to a technical difficulty with the auto-analyzer which ran out of reagents and gave initial values of zero.

Urinalysis revealed no unusual findings.

Pathology

I. Gross Necropsy

At necropsy, no compound related lesions were observed. A number of spontaneous lesions were noted in all groups. These findings included thickening of mammary areas, interdigital cysts, discolored lungs, mottled liver, kidney capsular adhesion, and corneal opacity.

Pathology

II. Organ Weight Data

Evaluations terminal body weights and organ-to-body weight ratios in the original metolachlor dog study received Dec. 11, 1979, was found to be unreliable. A comparison of the tables on pages 150-154 labeled as "Absolute and Relative Organ Weights, Terminal Sacrifice" with the tables pages 22-25, labeled as "Individual Body Weights" revealed many significant differences in terminal body weight versus week 26 body weights. The range of weights varied as much as 3.52 kg with many weights varying 1 or more kilograms. Ten weights differed by less than 0.5 kg and all but one weight

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On 1/21/80, Ciba-Geigy submitted an addendum to their 6 month dog study to answer questions related to the body weights and resultant questionable body weight ratios.

The addendum included the findings of two Ciba-Geigy toxicologists (J.M. Charles and J.T. Stevens) who went to IRDC to determine the cause of the unremarkable terminal weights. Laboratory records for weekly weighings, terminal body weights, necropsy records, food consumption data, diet preparation records, compound diet calculations, scale calibration records for scales used in both the necropsy room and for weekly weighings, clinical observation data, and a list of personnel involved in this study were also included in the submission.

The source of the problem was determined to be a faulty balance in the necropsy room used for the final weighings. This balance (manufactured by National Control, Inc. of Scope Inc., Serial#D785660) was different from the balances used for all the previous weekly weighings (Toledo balance Serial#9692). Calibration checks of the balance during each weekly weighing were included in the submission substantiating that weekly weights were valid.

The National Control balance in the necropsy room was designed to provide equal readings across the total surface of a large stainless steel pan, regardless of where a mass was applied. Two screws which secured the position of a central column beneath the pan had loosened during use. Since the balance was calibrated by placing the reference weight in the center, the calibration indicated normal functions, but when the dogs were weighed (off-centered) irregular terminal dog weights resulted and this problem therefore went undetected.

This balance was checked by Ciba-Geigy representatives when they visited IRDC and it was also found to work properly. They were told it had been repaired. In addition, they checked the digital balance used to weigh diets for food consumption estimates by using 0.5 kg and 2 kg reference weights and it was found to be accurate.

Food consumption and diet preparation data indicates animals were fed up to the day before sacrifice and then fasted overnight. This can therefore be ruled out as a contributing factor to the irregular terminal body weights. The addendum clearly indicated that the irregular values are due to the faulty necropsy room balance, which was discovered by IRDC personnel several months later (September 20, 1979).

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<u>Re-evaluation of the metolachlor 6-month dog feeding study using data supplied in the addendum of 1/21/80</u>

A. The following new data were submitted in this addendum:

1. Relative organ weights based upon week 26 weights taken on June 4, 1979, instead of the erroneous terminal weights taken 1-3 days later.

2. Organs-to-brain weight ratios

3. Values for some animals were inadvertently omitted from the IRDC submission (Attachments 5 and 6), so Ciba-Geigy submitted these under attachments 5A and 6A. They also included relative spleen, liver, and adrenal relative weights from the recovery animals that were not included by IRDC.

B. Review of addendum organ-to-body weight ratios, organ-to-brain weight ratios, and week 26 body weights.

Mean week 26 body weights were reduced in 300 and 1000 ppm males, but not at statistically significant levels, as follows:

	Control	100 PPM	300 PPM	1000 PPM
Males (kg)	14.48	13.55	12.83	12.5
Females (kg)	10.53	10.77	10.52	10.40

Males demonstrated a statistically significant increase in pituitary to body weight ratios at 100 ppm (0.54) with $p < 0.05$ and at 100 ppm (0.54) with $p < 0.01$, as compared to control males at 0.44. At 300 ppm, males actually showed a decrease in pituitary weight as compared to controls suggesting the effect at 100 ppm is questionable. Furthermore, there was no statistical significance in pituitary-to-brain weight ratios among any male test groups. One 300 ppm female was determined to have a cranio-pharyngeal cyst which greatly affected mean weights from that group, but this cyst is congenital and not related to treatment.

The thyroid-to-brain weight ratio was statistically significantly increased in 300 ppm females at 1.61 (with $p < 0.05$) as compared to controls at 1.22. Although not indicated as statistically significant, the 1,000 ppm level also showed an elevated value of 1.37. The thyroid to body weight ratio and mean weights (although not statistically significant were also elevated in the 300 and 1000 ppm females. When the 2 control recovery males were added to the 6 control males, for purposes of statistical comparison, mean thyroid weights of the 300 ppm group males were also significant ($p < 0.05$).

FEMALES	Control	100 PPM	300 PPM	1000 PPM
Thyroid (g)	0.94	0.84	1.26**	1.10
% body wt.	0.92	0.79	1.20	1.08
% brain wt.	1.22	1.07	1.61*	1.37

* significantly increased $p < 0.05$

** statistically significant ($p < 0.05$) when 2 recovery controls included in evaluation.

No thyroid effects were noted in males.

Heart weights were statistically reduced at $p < 0.05$ in females of the 1000 ppm group (Wilcoxin, Mann, Whitney Rank Sum Test).

In the males, a statistically significant decrease was determined in heart weights ($p < 0.05$) by both the t-test and the Wilcoxin, Mann, Whitney Rank Sum Test. This was not indicated as statistically significant in the IRDC revision nor in that submitted by Ciba-Geigy1/21/80, but was included in the 2/6/80 submission. Heart and heart-to-brain weight ratios were reduced in all other test levels as well, but not at significant levels.

MALES	Control	100 PPM	300 PPM	1000 PPM
heart (g)	110.89	101.37	94.13	94.98
% brain wt.	138.96	118.02	109.67*	121.77

* Statistically significant, $p < 0.05$

Spleen were reduced in males at 1000 ppm, although not at significant levels. In females, a reduction in spleen-to-brain weight ratio was significant at the 300 ppm level ($p < 0.05$, Wilcoxin, Mann, Whitney Rank Sum Test) and at the 1,000 ppm level, but not at a significant level. This statistically significant decrease in female spleen weight was not noted in the addendum data. Spleen is a unreliable indicator, and such findings likely relates to completeness of exsanguination at terminal sacrifice.

Although not indicated in the addendum submission, female liver-to-brain weight ratios were reduced at the 100 ppm dose level ($p < 0.05$, Wilcoxin, Mann, Whitney Rank Sum Test). No effects were evident in higher dose groups or in males and this finding is therefore not considered compound related.

Recovery Group Organ Weights

Since 1000 ppm recovery groups consisted of only 2 dogs/sex, statistical comparison to recovery controls is of little value.

After the 30 day recovery period elapsed, differences for a number of organs still existed in the 1000 ppm recovery animals. The only statistical difference noted was the reduction in spleen-to-brain weight ratio of 1000 ppm females ($p < 0.05$). Male liver weights and liver-to-brain weight ratios were reduced 15% and 13.7% respectively.

Male and female kidney weights and kidney-to-brain weight ratios decreased. Male gonad and brain weight ratios decreased 23.7% and 22.8% respectively. Male and female heart weights (17% and 30% respectively) and heart-to-brain weight ratios decreased.

Male thyroid weights and brain weight ratios decreased (39% and 37% respectively) as compared to controls.

The significance of these recovery weight differences is of extremely limited value due to the very few animals involved.

III. Histopathology

No lesions considered compound related were noted. Findings unrelated to compound administration included a craniopharyngeal cyst in the pituitary, focal parafollicular cell hyperplasia of the thyroid, mucroliths in medullary tubules of the kidneys, interstitial pneumonia and hypospermatogenesis in maturing dogs. Endometrial hyperplasia, which appeared to be dose related is likely due to a retarded maturation of the female dogs at the 300 ppm and 1000 ppm levels. No control or 100 ppm females demonstrated this finding, while 4 out of 6 300 ppm females and 5 out of 8 1000 ppm females demonstrated minimal to moderate diffuse endometrial hyperplasia.

Mammary hyperplasia in the acini and ductal structures was also observed in some control and test females which is indicative of proestrus or estrus.

Study Conclusions

All problems associated with this 6 month metolachlor dog study (originally submitted Dec. 11, 1979) have been resolved in 4 addendums submitted by the registrant. These problems include:

1. A faulty balance in the IRDC necropsy room negated any validity of terminal body weights and all organ-to-body weight ratios based upon them. New organ-to-body weight ratios have been submitted based upon the final weekly weighing measured several days earlier. Organ brain weight ratios have also been submitted.

2. Significant reductions in APTT values have been determined to be due to faulty methodology at IRDC.

3. The registrant has also submitted revised statistical analyses of organ weight data since the original analysis was determined to be incomplete. (Received 2/6/80)

4. Original report pages, not included in the submission of 12/11/79 have been received.

5. Missing individual animal data from the IRDC report have been supplied.

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The NOEL in this study has been determined to be 100 ppm.

A revised IRDC final report reflecting all study revisions and addendums including corrected statistical evaluations should be submitted by the registrant.

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

#188 DD

005504

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

May 28, 1986

SUBJECT: Metolachlor, Effect on Coagulation in a Six-Month Dog Study

TO: Metolachlor File
Toxicology Branch

FROM: Robert P. Zendzian PhD
Pharmacologist
Mission Support Staff
Toxicology Branch
HED (TS-769)

5/18/86

Action

Reevaluation of the effect of Metolachlor on coagulation as shown in the following study;

Jessup, D.C.; Estes, F.L.; Jefferson, N.D.; et al. (1979) 6-month chronic oral toxicity study in beagle dogs: IRDC No 382-054. (Unpublished study including addendum and AG-A No. 5358, received Dec 11, 1979 under 100-597, prepared by International Research and Development Corp., submitted by Ciba-Gigy Corp. Greensboro, NC; CDL:099116C)

Conclusion

In the first evaluation of this study statistically significant decreases in activated partial thromboplastin time (APTT) were observed in males and females. These values were attributed to 'error' in methodology. However, dispite extensive investigation, no information was obtained to support this conclusion. The decreases appear to be dose and time related with indications of a trend. This is particularly apparent when the data are converted to percent of concurrent control. Additional evaluation of the effects of Metolachlor on the clotting system of the dog are required to determine if this is a compound-related effect.

Discussion

The six month dog feeding study showed statistically significant decreases in activated partial thromboplastin time (APTT) in a dose and time related fashion in both sexes (table 1). Abnormalities in clotting seen in this test almost always consist of increases in clotting time. Decreases in this parimeter are rare and most texts do not mention the possibility. On this basis the initial reviewer of this study considered the effect to be a result of technique rather than compound-related. A fuller reevaluation of this study leads to the conclusion that the effect is most likely compound-related and must be further investigated.

The initial reviewer of this study noted the decreases in APTT and undertook an intensive investigation of the possible errors in methodology that could produce these results (Chitlik 1980). The following points, in order of their appearance in the memo, were established during this investigation.

1. The laboratory (IRDC) "had not observed shortened APTT values except in this study and that they were at a loss to explain it."

2. "There is a possibility of interference from elevated temperature and interference by unknown particulates."

3. "Dr Kahn (of IRDC) believes that 'this particular test cannnot be interpreted clinically based on shortened reaction time.'"

4. "He (Dr. Kahn) also indicated that APTT values are within the range based on other values obtained from other studies." An extensive discussion ensued on the use of historical control values from which it was concluded that properly obtained such values can be used.

5. Registrant toxicologists identified possible sources of error as;

"A. Optical interference induced by a technician pouring off supernatant and including some blood cells. (Not a likely cause)

B. If insufficient citrate were added, the clotting process could have been initiated prematurely. (Not considered likely problem)

C. The procedure used at IRDC did not limit the time between sample collection and analysis.

Memo, Chitlik, L.D., Proposed Tolerances for Residues of the Herbicide Metolachlor, Feb 7, 1980

D. Ignoring the significance of point 3 above, IRDC claimed that reported values were within the lower limit of the normal range and that the statistical significance is a Type I error (false positive)."

6. "we (Chitlik and Kahn) discussed the fact that APTT times for dogs at IRDC are several times shorter than human values and perhaps that IRDC was not abe (sic) to properly control processing time delays in the animal studies and obtain valid APTT normal range tolerances (ie - the IRDC range is 5.4 seconds while another laboratory referenced by Dr. Kahn, that of Dr. Hugh Lewis, had a range of 2.3 seconds."

7. "The question was raised by this reviewer (Chitlik) whether IRDC had considered not activating the thromboplastin times and whether the values would then be more reliable. Dr Kahn didn't answer this question at the time," or ever.

8. "Dr Kahn indicated that some findings were unusally low in this study and time intervals between obtaining the sample and the completion of the APTT were 'not rigidly controlled.'"

9. "The letter of Jack Norton, 1/28/80, Ciba-Geigy, also addressed the wider APTT normal range at IRDC and concluded that some variable is not controlled at IRDC and that it is likely due to delays in analysis of samples. --- the position of Ciba-Geigy is 'the reduced APTT values reported in this study are not meaningful in regard to the toxicity of metolachlor.'"

The EPA reviewer concluded;

"This reviewer agrees with the registrant that APTT values in this study are not related to a compound effect and are due to incorrect methodology."

The arguments advanced against the toxicological 'reality' of the APTT results fall into three catagories, 1) Problems with methodology, 2) rarity of the observation and 3) clinical significance or interpertation of the observation. Comments 2, 4, 5, 6, 7, 8 and 9 above deal with the methodology and will be addressed by first considering the test involved.

The APTT test was designed as an improvement on the partial thromboplastin time (PTT) test. The PTT test is a variation of the recalcification test designed as an improvement on whole blood clotting time by "providing optimal amounts of phospholipid and fully activating factors XII and XI." "The PTT is a more useful test than the recalcification time, but it does not eliminate the variable of partial activation of factors XII and XI." This activation occurs mainly by contact of the factors with the glass wall of the sample container.

Thus the degree of activation of factors XII and XI can be a function of the time between collection and assay. (Williams)

The ATPP test was designed "to circumvent this problem, a modification of the PTT has been introduced wherein some reagent capable of fully activating factors XII and XI is added to the plasma prior to recalcification". (Williams)

The major concern of possible technical errors in this study was attached to the 'variable' time between sample collection and analysis. In actuality the APTT test was designed to compensate for this variable within reasonable limits. If sample collection and analysis are performed in the same working day, time of holding samples should not be a problem. An experienced technician and one animal care man should be able to collect blood samples from the 56 dogs on this study in two hours. The samples are then taken to the lab and run as a batch. If the samples are collected in one order and run in the same order, the time between sample collection and analysis should be essentially the same for each sample. This 'if' appears to have been the procedure at IRDC.

A second concern was the variation of historical control APTT values at IRDC. Clotting tests are notorious for their variability. This is not of particular importance in clinical testing where one looks for increases of clotting time above the 'normal' range of values. To be clinically meaningful, that is to show a bleeding problem, these increases must be relatively large. In an experimental situation where one wishes to determine if a compound has any effect on clotting, test values must be compared with concurrent controls. It is the variability against the concurrent controls that is most important so that historical controls cannot be used for this type of determination (E. Zendzian personnal communication).

In all, none of the comments on methodology indicate a real problem and some of them show a lack of understanding of the APTT test.

The comment on the rarity of the observation, this was the only study to show it in the history of IRDC, indicates more that it is a real effect rather than a technical error. One can reasonably assume that the quality of performance of the APTT test at IRDC, whether good, bad or indifferent, is relatively consistant from day to day. Thus, one would expect lowered values to appear from time to time in a random manner. This is not true from study to study, as reported by Kahn, and does not appear to be true within this study. Eliminating the one-month withdrawal values as being on only two animals, the mean monthly control values are relatively consistant. The mean treatment values fall below the lowest of these

Williams, Chap 137, in Hematology, Williams, Beutler, Erslev and Lichtman, McGraw-Hill, 1983

values in seven incidences for the males and in five for the females. These lower values are not the result of a single very low individual value but rather three or four individual values per expermental group. Converting the values to percent of concurrent control (table 2) we see that the decreases tend to cluster in the intermediate and high dose groups toward the end of the study. This would not be expected for a random technical error.

Considering the clinical significance or interpertation of the observation is inappropriate for this type of study. This is a safety study in which we are attempting to identify any effects of the compound on the experimental animal not an effort to determine if the experimental animal is sick. The clinical significance becomes important only in subsequent steps where we consider the relation of the effect in the dog to a potential effect in man.

The following argument can be mustered that the decrease APTT observed in this study is compound-related.

1) The effect appears after three to four months of treatment with the first statistically significant effect at four months. 2) The effect increases with time and dose particularly in the males, which appear to be the most sensitive sex. 3) Although the decreases are small in magnitude they are proportionately large considering the initial position on the clotting curve. 4) The laboratory has never seen a decreased APTT in a dog study before this study.

The first two points are the basis for determining if an experimental observation is compound-related. Simply put, the more you dose the experimental animal, whether by time or magnitude, the more effect is expected. The fact that the effect observed is rare makes the case for compound-relation stronger.

The small size of the decrease in APTT is misleading as it conceals a relatively large change in 'activity' of coagulation factors. Coagulation in the dog is fast, both relatively and absolutely. Control APTT times in this study are 10-11 seconds which is normal. Therefore, the absolute ability to decrease clotting time is limited and the relative meaning of a small decrease is large. In addition, in clotting the relationship between coagulant activity as percent of maximum activity and clotting time in seconds is hyperbolic. "This relationship is such that small differences in clotting time represent major differences in activity when the clotting times are short, but they represent minor differences when the clotting times are long. Thus a change in the one-stage prothrombin time from 13 to 15 s may represent a decrease of 40 percent of the coagulant activity, while a change from 23

to 25 s may represent about 3 percent of the coagulant activity." (Williams).

As noted above the rarity of this observation and its clustering within the study support the observtion of decreased APTT being a compound-related response rather than being a technical error.

Considering the rarity of this observation, the potential for harm of increased intravascular coagulation and the questions raised as to its 'reality', the Agency is requiring that the Registrant perform studies in the dog to investigate the effect(s) of Metolachlor on coagulation system.

Table 1. Activated partial thromboplastin time (seconds) in dogs.

MALES	Month	Control	100 ppm	300 ppm	1000 ppm
	pretest	15.0	15.0	15.0	16.0
	1	12.0	12.0	12.0	12.0
	2	11.0	11.1	11.1	11.3
	3	11.5	10.5	11.6	11.6
	4	10.9	10.5	10.5	11.2
5		11.6	11.0	10.0**	10.1**
	6	11.5	12.3	9.6**	9.7**
	7-WD	9.4			10.0

FEMALES	Month	Control	100 ppm	300 ppm	1000 ppm
	pretest	15.0	14.0	15.0	16.0
	1	12.0	12.0	12.0	17.0
	2	11.2	11.3	11.2	11.3
	3	11.4	10.7	11.5	11.2
	4	11.0	10.4*	11.8	10.9
	5	10.9	11.0	11.0	9.8**
	6	11.3	11.6	9.9*	10.3
	7-WD	11.0			9.3**

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

Table 2. Activated partial thromboplastin time in dogs expressed as percent of concurrent control.

MALES	Month	Control	100 ppm	300 ppm	1000 ppm
	pretest	100	100	100	107
	1	100	100	100	100
	2	100	101	101	103
	3	100	91	101	101
	4	100	96	96	103
	5	100	95	86**	87**
	6	100	107	83**	84**
	7-WD	100			106

FEMALES	Month	Control	100 ppm	300 ppm	1000 ppm
	pretest	100	93	100	107
	1	100	100	100	142
	2	100	101	100	101
	3	100	94	101	98
	4	100	95*	107	99
	5	100	101	101	90**
	6	100	103	88*	91
	7-WD	100			85**

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

END