10-21-85

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

004725

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Additional Toxicology Data for Metolachlor.
EPA ID No. 100-587, Tox. PN #44; CASWELL #188DD

TO: Richard Mountfort (23)
Registration Division (TS-767)

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

THRU: Laurence D. Chitlik, DABT W. Teller for L. Chitlik 10-11-85
Head, Section V
TOX/HED (TS-769)
and
Theodore M. Farber, Ph.D. 10/21/85
Chief, Toxicology Branch
Hazard Evaluation Division (TS-679)

Action Requested

Review submission by Ciba-Geigy which consisted of: (1) nasal turbinate examinations from the rat 2-year chronic feeding study; (2) an in vivo micronucleus test in Chinese hamsters; (3) a DNA repair assay in human fibroblasts; (4) a DNA repair assay in rat hepatocytes; (5) data for the stability of the test compound; and (6) a letter from the study director of the mouse oncogenicity study describing the method of sacrifice in that study.

Recommendations

1) The examinations of nasal turbinates of control and high dose rats from the two year feeding study yielded data that were suggestive of an oncogenic response at this site in treated males. The observed incidence of adenocarcinoma was 0/67 control and 2/69 high dose, and for fibrosarcoma, incidences of 0/67 control and 1/69 high dose were observed. Although these tumors arise from histogenetically distinct cell types, the etiology of tumor formation may be the same, i.e. a toxic effect of metolachlor. If the incidences are combined, a cumulative incidence for nasal malignancy of 3/69 high dose vs. 0/67 control is obtained. These values approached statistical significance ($p<0.1$ by the Chi-square test).

Therefore, although these data alone are not convincing evidence of oncogenicity, when considered with the findings of liver neoplasia identified in the review of the original study (memo, Burin to Mountfort, 12-14-84), they are further evidence for the oncogenicity of metolachlor in the rat.

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Recommendations (con't)

2) The Chinese hamster micronucleus test (study #831498) was classified as Inconclusive because no data were submitted to demonstrate that the test material reached the target tissue, the bone marrow. Data from the range-finding toxicity study were not submitted. No evidence of mutagenicity was observed in this study.

The Registrant is requested to submit, at a minimum, range-finding data and evidence that the test article reaches the target tissue examined in this study, the bone marrow. Alternatively, the Registrant may wish to consider an in vitro assay for cytogenetic effects.

3) The DNA repair test in human fibroblasts (study #831499) was classified as Unacceptable. No range-finding data or evidence of cytotoxicity were submitted to support the selection of the concentrations of metolachlor that were tested, nor was the effect of metabolic activation assessed. Current guidelines require that chemicals be tested to the limits of cytotoxicity or solubility. No evidence of mutagenicity was noted in this study.

The Registrant is requested to submit either range-finding data or evidence that the test article was tested to the limits of cytotoxicity in this study.

4) The DNA repair assay in rat hepatocytes (study #831497) was also reported as negative, and was similarly classified as Unacceptable due to the lack of data demonstrating the adequacy of the concentrations of metolachlor that were tested.

The Registrant is requested to submit either range-finding data or evidence that the test article was tested to the limits of cytotoxicity in this study.

5) In response to questions raised in the review of the mouse oncogenicity study (#79020, memo, Saunders to Mountfort, 8-3-84), the results of stability studies conducted on the test article and the method of sacrifice of test animals were submitted.

The stability study demonstrated that the concentration of test material used in the mouse study remained stable over the course of study.

The description of the method of sacrifice indicated that mice were "euthanitized" [sic] with ether. In the experience of this reviewer, a more common procedure is induction of anesthesia with ether, followed by decapitation or exsanguination as the method of sacrifice. Sacrifice by means of ether overdose seems an unlikely maneuver due to the length of exposure necessary, and the known effects of excessive ether exposure on the histology of the lung. In any case, no questionable findings were noted in the lungs of control or treated mice. Therefore, the method of sacrifice did not appear to have any significant effect on the results of the study.

The two issues raised in the review of the mouse oncogenicity study have been satisfactorily addressed, and it is recommended that the classification of this study be upgraded to Core-Guidelines status.

Data Evaluation Record 004725

Study Type: Addenum to the two year rat chronic feeding study.

Study Identification: "Amendment No. 1 to Final Report: Microscopic Evaluation of Nasal Turbinates."

Lab. performing study: Hazelton Laboratories America, Inc.
Life Sciences Division
Madison, Wisconsin 53704

Sponsor: Ciba-Geigy Corporation
Agricultural Division
Greensboro, N.C.

Study no.: 80030
Accession no.: 258390
Report date: April 29, 1985
Submitted to EPA: 6/14/85
Study author: Merrill Tisdel

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

Approved By: Louis Kasza, DVM, Ph.D.
Pathologist, Toxicology Branch
Hazard Evaluation Division (TS-769)

Conclusions: Adenocarcinoma of the nasal turbinates was noted in 0/67 control and 2/69 high dose (3000 ppm) male rats. Fibrosarcoma of the nasal tissue was noted in 0/67 control and 1/69 high dose males. Neither lesion was noted in control or high dose females. Although not statistically significant, the combined incidences of nasal malignancies are suggestive of an oncogenic response in the nasal turbinates of treated males. When considered with the previously identified findings of liver tumors in this study, these data are additional evidence that metolachlor is oncogenic in rats.

Classification: Core-Minimum

Background

The examination of nasal turbinates was apparently requested by the sponsor because "a high incidence of adenomas in the nasal turbinates was reported in two rat chronic studies from a closely related chloroacetanilide".

Materials and Methods

The materials and methods used in the two year rat feeding study have been previously reviewed (memo, Burin to Mountfort, 12-14-83). In the present study, three sections of the nasal cavity were taken from each control and high dose animal available. The first section was taken from "just behind the incisors", section 2 was taken "just anterior to the first molar", and section 3 was taken "approximately midway through the second molar".

Sixty-seven control males and females, and 69 high dose (3000 ppm) males and females were examined.

Results

Data were submitted as summary incidence tables and as individual animal findings.

An apparent treatment-related increase in the incidence of adenocarcinoma of the nasal turbinates was noted in males (Table 1, photocopied from the study report). This lesion was not noted in 67 control animals, but was observed in 2/69 high dose males. A single fibrosarcoma was also noted in an additional high dose male, but was not observed in control males. Neither lesion was observed in control or treated females.

No relationship to survival was apparent, as the adenocarcinomas were noted in a terminal sacrifice male (#7503) and a moribund sacrifice male (#7551, date and reason for sacrifice not stated). The fibrosarcoma was noted in a male (#7509) that died on test (date and cause of death not specified).

Other non-neoplastic lesions such as congestion, hemorrhage and acute inflammation were noted with similar frequency in control and treated animals (Table 2, photocopied from the study report). A possible treatment-related increase in the incidence of fibrous osteodystrophy was noted in treated males: 0/67 control vs. 3/69 high dose, observed in animals other than those observed to have tumors. A similar effect was not apparent in females (1/67 control vs. 0/69 high dose).

Table 1

Summary of Neoplasms[a]

Group	Male		Female	
	1	4	1	4
No. Animals Examined	67	69	67	69
Neoplasm Incidence				
Adenocarcinoma	0	1	0	0
Adenocarcinoma (gland)*	0	1	0	0
Adenomatous polyp	1	0	0	0
Fibrosarcoma	0	1	0	0
Odontoma	0	0	1	0
Papilloma, squamous cell	0	0	0	1

* Tumor appears to arise in either lacrimal gland or glands of nasal submucosa.

[a]data photocopied from submitted study report.

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—LETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY
MADISON, WISCONSIN 53707

Table 2 [a]

PRINTED: 30-APR-85
PAGE: 1

INCIDENCE SUMMARY

STUDY NUMBER: 80030A

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=D,M,1,2,T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	70	0	0	70	70	0	0	70
** TOP OF LIST **									
NASAL PASSAGE 1 (NP0)	NUMBER EXAMINED:	67	0	0	69	67	0	0	69
	NOT REMARKABLE:	42	0	0	39	42	0	0	46
--AUTOLYSIS		7	0	0	8	5	0	0	4
--CYST, NOS		0	0	0	1	0	0	0	1
--DENTAL FRACTURE		0	0	0	2	6	0	0	3
--CONGESTION		7	0	0	11	11	0	0	2
--HEMORRHAGE		9	0	0	8	7	0	0	11
--INFLAMMATION, ACUTE SUPPURATIVE		8	0	0	6	6	0	0	5
--INFLAMMATION, CHRONIC		2	0	0	3	6	0	0	1
--FIBROUS OSTEODYSTROPHY		0	0	0	3	1	0	0	0
--B-ODONTOMA		0	0	0	0	1	0	0	0
--M-ADENOCARCINOMA, TUMOR APPEARS TO ARISE IN EITHER LACRIMAL GLAND OR GLANDS OF NASAL SUBMUCOSA		0	0	0	1	0	0	0	0
--M-FIBROSARCOMA		0	0	0	1	0	0	0	0
NASAL PASSAGE 2 (NP1)	NUMBER EXAMINED:	67	0	0	69	67	0	0	69
	NOT REMARKABLE:	50	0	0	46	48	0	0	58
--AUTOLYSIS		9	0	0	6	3	0	0	4
--DENTAL FRACTURE		0	0	0	1	5	0	0	0
--CONGESTION		7	0	0	7	8	0	0	3
--HEMORRHAGE		3	0	0	5	1	0	0	5
--INFLAMMATION, ACUTE SUPPURATIVE		2	0	0	3	6	0	0	1
--INFLAMMATION, CHRONIC		1	0	0	0	2	0	0	0
--FIBROUS OSTEODYSTROPHY		0	0	0	3	1	0	0	0
--B-ODONTOMA		0	0	0	0	1	0	0	0
--B-ADENOMATOUS POLYP		1	0	0	0	0	0	0	0
--M-ADENOCARCINOMA		0	0	0	2	0	0	0	0
--M-FIBROSARCOMA		0	0	0	1	0	0	0	0
NASAL PASSAGE 3 (NP2)	NUMBER EXAMINED:	67	0	0	69	67	0	0	69
	NOT REMARKABLE:	55	0	0	51	59	0	0	64
--AUTOLYSIS		7	0	0	5	2	0	0	2

** CONTINUED ON NEXT PAGE **

[a] Table photocopied from submitted study report.

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...LETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY
MADISON, WISCONSIN 53707

Table 2 (Continued)[a]

INCIDENCE SUMMARY

STUDY NUMBER: 800308

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL,WEEKS=ALL
DEATH=D,M,1,2,T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	70	0	0	70	70	0	0	70
** FROM PREVIOUS PAGE **									
NASAL PASSAGE 3 (NP2)	NUMBER EXAMINED:	67	0	0	69	67	0	0	69
	NOT REMARKABLE:	55	0	0	51	59	0	0	64
--CONGESTION		3	0	0	4	3	0	0	0
--HEMORRHAGE		3	0	0	5	2	0	0	2
--INFLAMMATION, CHRONIC		1	0	0	0	2	0	0	0
--FIBROUS OSTEODYSTROPHY		0	0	0	3	1	0	0	0
--B-PAPILLOMA, SQUAMOUS CELL		0	0	0	0	0	0	0	1
--M-ADENOCARCINOMA		0	0	0	1	0	0	0	0
--M-FIBROSARCOMA		0	0	0	1	0	0	0	0
** END OF LIST **									

[a] Table photocopied from submitted study report.

Discussion

These data are suggestive of an oncogenic response in the nasal tissues of male rats. Although adenocarcinoma and fibrosarcoma cannot be considered as histogenetically related, it is possible that these two tumor types arose as a result of insult by the test article. If the incidences of the two tumor types are combined, the overall incidence of malignant neoplasms in the nasal turbinates becomes 0/67 control and 3/69 high dose. These values approach statistical significance ($p < 0.1$ by Chi-Square test). The finding of an adenomatous polyp in a single control male is not considered significant as this type of lesion is not neoplastic. Hyperplasia of nasal tissues was not reported for any of the animals, therefore the apparent oncogenic response cannot be related to any pre-neoplastic changes.

Therefore, these data add to the weight of the evidence that metolachlor is oncogenic in rats. A statistically significant increase in neoplasms of the liver in females was previously identified in Mr. Burin's review of the original study. A similar effect was suggested in males, although the response was not statistically significant. The present data suggest that males (but not females) are subject to induction of malignant neoplasms of the nasal turbinate by metolachlor.

The apparent findings of fibrous osteodystrophy in treated males may also be related to treatment, however the etiology of this lesion is uncertain in the present study. According to Veterinary Pathology (T. Jones and R. Hunt, Lea & Febiger, Philadelphia, 1983, pages 1167-1173), this lesion is generally the result of hyperparathyroidism, which may be primary or secondary to hypocalcemia. The original review of this study by Mr. Burin did not note any effects on the parathyroid nor on blood calcium, although some effects on renal tubular epithelium were noted.

Classification: Core-Minimum

Data Evaluation Record 004725

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.
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 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.

METOLACHLOR TOXICOLOGY REVIEW

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___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

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___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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Data Evaluation Record

004725

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.

METOLACHLOR TOXICOLOGY REVIEW

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Data Evaluation Record

004725

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)

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.

004725

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.

METOLACHLOR TOXICOLOGY REVIEW

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