US EPA ARCHIVE DOCUMENT

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

4/17/91

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

**MEMORANDUM**

SUBJECT: Second Peer Review of Metolachlor

FROM: Gary Burin, Ph.D., D.A.B.T
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Richard Mountfort
Product Manager #23
Registration Division (H7505C)

The Health Effects Division Peer Review Committee (PRC) met on April 17, 1991 to discuss and evaluate the weight-of-the-evidence on metolachlor with particular reference to its carcinogenic potential. This was the second occasion that metolachlor was evaluated by the PRC.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp _____

William L. Burnam _____

Reto Engler _____

Karl Baetcke _____

Marcia Van Gemert _____

Esther Rinde _____

Kerry Dearfield _____

Marion Copley _____

Robert Beliles _____

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Julie Du _____

Yin-Tak Woo _____

Hugh Pettigrew _____

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Stephen Dapson _____

M. Ioannou _____

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

William Sette _____

George Ghali _____

Richard Hill _____

Jean Parker _____

Jack Quest _____

4. Other Attendees: Gary Burin (HED), Bernice Fisher (HED), Carmine Pellosie (OPTS).

B. Material Reviewed:

The peer review package was prepared by Dr. Stephen Dapson. The material available for review consisted of DER's, one-liners, and other data summaries prepared by HED scientists. An Executive Summary was submitted by the manufacturer and was included in the peer review package. The material reviewed is attached to the file copy of this report. Metolachlor was previously reviewed by the PRC on May 30, 1985 and the data base available to that meeting (and the conclusion derived by the PRC from that data base) was made available to the present PRC.

C. Background Information:

Metolachlor is the common name for 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide, one of the family of chloracetanilides. The CAS number is

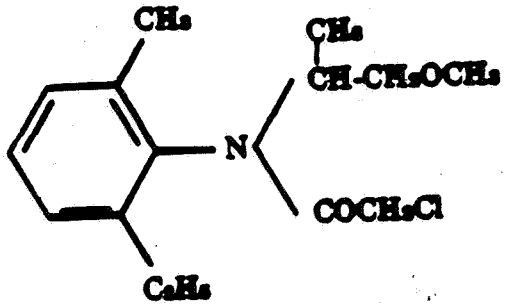
51218-45-2, EPA Pesticide Chemical Code is 108801, and the Toxicology Chemical Number is 188DD.

Metolachlor is a selective herbicide registered for use (CFR 180.368) for the control of most annual grasses and certain broadleaf weeds in corn, soybeans, peanuts, grain sorghum, potatoes, pod crops, cotton, safflower, and woody ornamentals.

The previous PRC meeting concluded that data available for metolachlor provided "weak evidence of carcinogenicity", but did not assign metolachlor a classification for carcinogenicity. It recommended that a metabolism study and a battery of mutagenicity studies be conducted according to EPA guidelines and submitted for review.

Structure of:

Metolachlor




D. Evaluation of Carcinogenicity Evidence:

1. Chronic Feeding Study in Rats. Industrial Biotest Laboratory Study No. 622-07925. Submitted 2/9/79 (EPA validation- Supplementary Data). MRID#244166, 099628, 099626, 070048.

In the IBT study in Charles River rats (No. 622-07925), the following incidence of liver hyperplastic (i.e., neoplastic) nodules and carcinomas occurred in female albino CD Charles River rats receiving metolachlor in the diet for 2 years.

| Dose (ppm) | 0 | 30 | 300 | 1000 | 3000 |
|---|---|---|---|---|---|
| Number of Females Examined (final sacrifice) | 54 | 58 | 60 | 60 | 60 |
| Hypertrophic-Hyperplastic Nodules | 1 | 1 | 3 | 3 | 9 |
| Carcinoma | 0 | 0 | 0 | 0 | 2 |
| Total (No. Animals with primary liver tumors) | 3 | 1 | 3 | 3 | 11 |

The increase in hypertrophic-hyperplastic nodules (i.e. neoplastic nodules) and hepatocellular carcinomas found in

high dose female rats was considered to be compound-related. A small increase was also observed in the incidence of cholangioma in high dose female animals (6 versus 2 in each of the other treated groups and controls). These were the only treatment-related carcinogenic responses 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 Core-Supplementary Data due to inadequate clinical chemistry determinations and dietary preparation records.

2. Chronic Feeding Study in Rats, Hazleton-Raltech Study No. 80030, submitted May 2, 1983. MRID# 250369, 250370, 250375, 245958, 258390.

In the second study in the rat, the following incidence of liver neoplastic nodules and carcinomas occurred in female CD-Crl:CD (SD)BR rats receiving metolachlor in the feed for 2 years.

| 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 liver neoplasia | 0 | 1 | 2 | 7** |

* = ($p < 0.05$) ** = ($p < 0.01$)

A statistically significant increase of hepatic neoplasia was found in high dose females at terminal sacrifice. This was the only carcinogenic 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 for the control, low, mid and high dose, respectively). The survival of the animals at 24 months was 54%, 57%, 42% and 57% for the control, low, mid and high dose groups, respectively. This study was classified as Core Minimum Data. This study was considered to be adequate for the evaluation of carcinogenicity.

Additional data were submitted relating to nasal turbinate examinations from the rat 2-year feeding study (Hazleton Laboratories America, Inc., Study No, 80030, 4/29/85). Adenocarcinomas of the nasal turbinates were noted in 0/67 control and 2/69 high dose (3000 ppm) male rats. Fibrosarcomas of the nasal tissue were noted in 0/67 control

and 1/69 high dose males. Neither lesion was noted in animals from the control group, at other dose levels in males or in treated females.

3. Mouse Carcinogenicity Study, Industrial Biotest Laboratories, Study No. 622-07925 (validated by USEPA). Submitted 12/15/1977.

A 2-year mouse study (No. 622-07925) using dietary levels of metolachlor of 0, 30, 1000 and 3000 ppm was classified as Core Minimum Data. No carcinogenic effects were noted. The PRC did not raise any issues concerning the results of this study.

4. Carcinogenicity Study in the Mouse. Hazleton-Raltech, Inc. Study No. 79020, submitted 8/13/1982. MRID# 248722.

A 2-year mouse study (No. 79020) using dietary levels of metolachlor of 0, 300, 1000 and 3000 ppm was reviewed and classified as Core Minimum Data. No carcinogenic effects were noted. The highest dose level tested produced a reduction in weight gain ($p < 0.05$) in male and female mice indicating that the chemical was tested at an adequately high dose to assess carcinogenic potential. A slight reduction in survival also was noted in high dose females (34.6% survival to end of test versus 53.8% controls, 38.5% low dose and 46.2% mid dose) which might have been related to Sendai virus infections early in the study.

E. Additional Toxicology Data on:

1. Metabolism

A total of 89.9% of the administered radioactivity was found to be excreted over 3 days in the CD-Crl:CD (SD)BR rat, and elimination of label was about equal between urine and feces (Ciba-Geigy Study No. 26/1). The pooled feces samples from days 1 and 2 accounted for about 46% of the administered radioactivity.

A subsequent metabolism study in the rat utilized a single low (1.5 mg/kg), a single high (300 mg/kg), and repeated low (1.5 mg/kg/day for 15 days) oral doses of metolachlor (Ciba-Geigy Study No. ABR-8611). Metolachlor was readily absorbed and excreted by male and female rats. Approximately 48 to 64% of the radiolabel was recovered within 7 days. Low levels of

radioactivity were found in the tissues of all animals at 7 days postdosing. This study did not satisfy the guideline requirement for a metabolism study.

2. Genotoxicity

Two genotoxicity studies were available to the previous PRC, a mouse dominant lethal study and a *Salmonella* assay. Both tests were considered to be acceptable at the time of the meeting and no genotoxic effects were found in either study. Subsequent review of the dominant lethal assay determined that the study was unacceptable because the mating period was too short and no toxicity was observed.

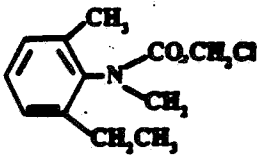
Several additional genotoxicity studies have since been made available to the EPA. A micronucleus test in Chinese hamsters (Ciba-Geigy Limited, Study No. 831498, 10/84) found no effect of treatment. Two primary DNA damage/repair assays (Ciba-Geigy Limited, Study No. 831499 and 831497, 11/84) were considered to be unacceptable. The first of these, a primary rat hepatocyte assay, was not acceptable because the incubation time was too short. The human fibroblast assay was unacceptable because cells were not inhibited at S-phase and because no activation was utilized. An *in vitro* gene mutation assay using mouse lymphoma cells (Ciba-Geigy Ltd., Study No. 831500, 12/5/84) did not yield a mutagenic response (in both the presence and absence of a metabolic activator). These studies satisfy 2 of the 3 categories for genotoxicity testing (gene mutations and structural chromosomal aberrations). A data gap exists for the other genotoxic effects category.

3. Developmental and Reproductive Effects

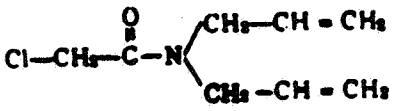
Metolachlor did not produce developmental or maternal toxicity (highest dose tested 360 mg/kg/day) in a teratology study in rats (Ciba-Geigy Ltd.). Metolachlor did not produce developmental toxicity at the highest dose tested (360 mg/kg/day) in rabbits, but produced maternal toxicity (lacrimation, miosis, decreased food consumption, and reduced body weights on gestation day 12 and 18) at this dose level (Argus Laboratories). A 2-generation reproduction study in rats produced reduced pup weights and parental food consumption at the highest dose level (1000 ppm) (Toxigenics Laboratories). A 3-generation reproduction study in rats found that the highest dose level tested (1000 ppm) was a NOEL (IBTL, Core Supplementary Data).

4. Structure-Activity Correlations

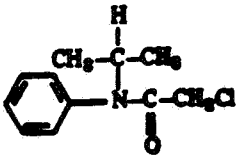
Metolachlor is structurally related to Acetochlor, Alachlor, Allidochlor, Butachlor, and Propachlor.



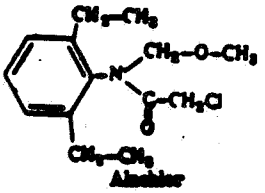
Acetochlor.



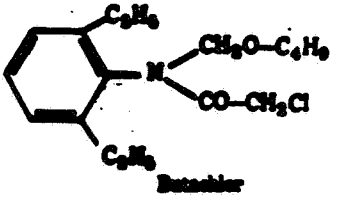
Allidochlor



Propachlor



Alachlor



Butachlor

Acetochlor has been classified as a Group B2 Carcinogen (Probable Human Carcinogen) by the PRC and the FIFRA Scientific Advisory Panel (SAP). This was based on the evidence that acetochlor caused an increased incidence of benign and malignant tumors at multiple sites in Sprague-Dawley rats (papillary adenomas of the nasal turbinates in both sexes; hepatocellular carcinomas in both sexes and thyroid follicular cell adenomas in males) and an increased incidence of benign and malignant tumors at multiple sites in Swiss albino CD-1 mice (hepatocellular carcinoma in both sexes; lung carcinomas in females; uterine histiocytic sarcoma and benign ovarian tumors in females; kidney adenomas in females). Acetochlor caused point mutations in a Chinese hamster ovary test both with and without metabolic activation, gene mutations in the mouse lymphoma assay (with activation), aberrations in human lymphocytes and unscheduled DNA synthesis (UDS) in an in vivo/in vitro UDS assay. There were no mutagenic effects in gene mutation tests in bacteria, a micronucleus assay inmice, an in vivo cytogenetics assay in rats and an unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes in vitro.

The PRC has classified Alachlor as a Group B2 carcinogen. In a dietary administration study in rats, nasal turbinate tumors and squamous cell tumors of the stomach were found in both sexes as well as thyroid follicular adenomas in males. In a dietary administration study in mice, there was an increased incidence of lung tumors in females. Alachlor was positive in one Salmonella assay (and negative in 4 others) and in a DNA damage/repair (UDS) assay, while it was negative in other bacterial assays, in vitro cytogenetics, and an CHO/HGPRT assay.

Allidochlor has no acceptable chronic or mutagenicity studies.

Butachlor is carcinogenic in rats, inducing stomach tumors in females in a dietary administration study. It was weakly mutagenic in one Salmonella assay, and negative in a rec assay

and for reversion.

Propachlor showed evidence of an increased incidence of follicular cell tumors of the thyroid and ovarian neoplasia in rats; however, this study did not test at high enough levels to adequately assess the carcinogenic potential of Propachlor. A carcinogenicity study in mice did not test at high enough levels to adequately assess carcinogenicity potential. Propachlor was positive in a chromosome aberration assay, and suggestive of a positive response in a CHO/HGPRT assay. It was negative in a rat bone marrow cytogenetics assay and in an in vitro UDS assay.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on metolachlor to be of importance in a weight-of-the-evidence determination of carcinogenic potential:

1. Metolachlor was found to induce hepatic neoplasia in female rats in two separate studies at a dose level of 3000 ppm. Most of the tumors which were induced by metolachlor were benign.

2. The small number of nasal turbinate tumors at the high dose level in the second rat study (2 adenocarcinomas and 1 fibrosarcoma) are suggestive of a response at that site although not, standing alone, convincing evidence of carcinogenicity. Nasal turbinate tumors are considered to be rare in oral carcinogenicity bioassays but have been associated with the dietary administration of two analogs (acetochlor and alachlor).

3. Although no statistically significant increase in neoplasia was observed in male rats, there was a positive trend for hepatic neoplastic nodules.

4. No increase in the incidence of neoplasia was associated with metolachlor administration in mice.

5. The limited genotoxicity data for metolachlor are negative.

6. Metolachlor is structurally related to several compounds which induce neoplasia in rats and/or mice.

G. Classification of Carcinogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered. The Peer

Review Committee agreed that metolachlor should be classified as Group C, a Possible Human Carcinogen, and recommended that a linear low dose extrapolation model be applied to the incidence of hepatic neoplasia observed in the rat for the purpose of risk characterization. This recommendation was based on the replication of the finding of hepatic neoplasia in female rats and the apparent induction of a small number of nasal turbinate tumors, an uncommon tumor induced by certain analogs of metolachlor, at the high dose level in the second rat study. Application of a linear low dose extrapolation model will facilitate comparison of the cancer potency of metolachlor with other members of this class of herbicide.

## H. Recommendations

An *in vivo*/*in vitro* unscheduled DNA synthesis assay is recommended to fulfill the data gap for genotoxicity testing.

CASWELL FILE

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

NOV 9 1987

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Company Response to EPA Reviews of Metolachlor; Miscellaneous Data. TOX PN #1878; Caswell #188DD. Accession Nos. 262712, 262713.

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

FROM: D. Stephen Saunders, Ph.D.
Hazard Evaluation Division (TS-769C) DSS 11-3-87

THRU: Quang Bui, Ph.D. Quang Bui 11-4-87
Head, Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

WSBun 11/5/87

Action Requested

Review company response to previous EPA review of nasal turbinate data from the 2-year feeding study in rats; review miscellaneous mutagenicity and metabolism data.

Recommendations

1. Toxicology Branch's conclusion regarding the significance of nasal turbinate tumors identified in the 2-year feeding study in rats (study #80030) remains unchanged from our previous evaluation (memo Saunders to Mountfort, 10-10-85): "The examinations of nasal turbinates of...rats are suggestive (emphasis added) of an oncogenic response at this site in treated males... 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, they are further evidence for the oncogenicity of metolachlor in the rat."

This compound has been previously classified as a Category C carcinogen by the Toxicology Branch Peer Review Committee (memo Engler to Mountfort, 8-23-85). This committee will determine whether the nasal turbinate data warrant any change in this classification.

2. Toxicology Branch agrees that the apparent increase in the incidence of "testicular atrophy" in male rats that died on test in the 2-year feeding study is of doubtful toxicological significance. This finding was not present at final sacrifice, and historical control data demonstrate that this finding is relatively common in rats. Therefore, this finding should not be considered as a basis for the ADI. The NOEL for the 2-year feeding study in rats is now 300 ppm, based on decreased body weight gain in rats fed 3000 ppm, the highest dose tested.

A cursory review of the data base for this chemical indicates that the lowest NOEL is now obtained in the 6-month feeding study in dogs. Accordingly, the ADI is tentatively established as 0.025 mg/kg, based on the NOEL of 100 ppm (equivalent to 2.5 mg/kg/day) in the dog study and a 100-fold safety factor. A final determination regarding the ADI will be made by the Toxicology Branch and Agency Reference Dose Committees.

3. The Registrant's explanations of the dose selection procedures in the chinese hamster micronucleus test (study #831498), the DNA repair test in fibroblasts (study #831499), and the DNA repair test in hepatocytes (study #831497) are satisfactory, and accordingly these studies are re-classified as Acceptable.

The mouse lymphoma cell gene mutation assay (study #831500) included in the present submission was classified as Acceptable. Metolachlor was not mutagenic in this assay.

4. The submitted metabolism data provided a revised proposed metabolic pathway. The study was classified as Core-Minimum data, however does not satisfy the metabolism data requirements unless considered with previously submitted data.

Discussion

1. The Registrant questioned several aspects of our previous review of the nasal turbinate data submitted for the 2-year feeding study in rats. The data originally submitted included the results of examinations only in control and high-dose animals. In the present submission, the results of examinations in the low- and mid-dose groups have been included. The points raised by the Registrant will be addressed individually.

(A) Ciba-Geigy: The combination of adenocarcinoma and fibrosarcoma "is not an appropriate combination according to" the EPA Standard Evaluation Procedure (SEP) for chronic toxicity/oncogenicity studies. "It is not clear why the reviewer combined these tumor types."

EPA Response: We clearly noted in our review of 10/10/85 that these tumors arose from histogenetically distinct cell types, but speculated that these tumors may arise from a common etiology, i.e. a toxic insult of metolachlor. This effort was in part due to the small amount of data available. The Registrant has now provided the results from the low and mid-dose groups, and so more sensitive statistical methods can be used based solely on the incidence of adenocarcinoma (see below).

(B) Ciba-Geigy: The registrant claims that EPA has failed to combine appropriate tumor types in its analysis. Specifically, it is claimed that the incidence of nasal polyp in the control group should be compared with that of adenocarcinoma in the high dose group when calculating statistical significance.

EPA Response: We do not agree that nasal polyps are neoplastic lesions. Although the single reference cited by the registrant seems to imply that this lesion should be considered as neoplastic (1), authoritative textbooks in the field of pathology area do not support this view.

In Veterinary Pathology (2), it is noted that "Nasal polyps (polypi) are new growths which resemble true neoplasms.... [They have] an inflammatory pathogenesis which puts polypi in the same category as the granulation tissue of wound healing....They have to be differentiated from....true neoplasms, which are encountered rarely." Similarly, Moulton (3) states: "A nasal polyp...is the result of hypertrophy of the mucous membrane or exuberant proliferation of fibrous connective tissue. It is not to be confused with pedunculated fibroma or papilloma, which are true neoplasms". A similar interpretation is offered by Robbins (4): "The familiar nasal 'polyp' is not in reality a true neoplasm. These polyps represent focal accumulations of edema fluid accompanied by some hyperplasia of submucosal connective tissue. As such, they are not neoplastic but rather inflammatory in nature."

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(1) Pour, P., Stanton, M.F., Kuschner, M., Laskin, S. and Shabad, L.M. (1973). Tumours of the respiratory tract. In: Pathology of Tumours in Laboratory Animals, V. 1; V.S. Turusov, ed. IARC Scientific Publications, no. 5-6, Lyon.

(2) Jones, T.C. and Hunt, R.D., 1983. The respiratory system. In: Veterinary Pathology, fifth edition, Lea & Febiger, Philadelphia (p. 1210).

(3) Moulton, J.E., 1978. Tumors of the respiratory tract. In: Tumors in Domestic Animals, second edition, J.E. Moulton, (ed.); University of California Press, Berkeley (p. 215).

(4) Robbins, S.L., 1974. Pathologic Basis of Disease, W.B. Saunders Co., Philadelphia (p. 847).

Finally, the registrant implies that the EPA SEP recommends combination of nasal polyps and adenocarcinoma. This contention is absolutely incorrect. The registrant is referred to page 94 of that document for clarification.

Toxicology Branch concludes that the inclusion of nasal polyps with the incidence of adenocarcinoma is unsupported.

(C) Ciba-Geigy: The registrant disagrees with the statistical analysis conducted in our original review, and requests "an explanation for the reasoning underlying the conclusions ultimately reached".

The registrant summarizes their position with the statement: "The low incidence and distribution of nasal passage tumors in this study are not considered to be statistically-significant or biologically meaningful... Therefore, it is concluded that feeding metolachlor in the diet for two years had no effect on the incidence of nasal passage tumors."

EPA Response: We agree that our original approach was unconventional. The reason for our approach was simply due to our relative lack of experience with this tumor type, and the small amount of data submitted by the registrant on which to base a decision. Therefore, we chose to be conservative, rather than completely doctrinaire with regard to the SEP, in view of our uncertainty regarding this particular tumor type.

In any case, with the submission of the results of examinations of nasal cavities in the intermediate dose groups, we now have available a complete data set on which to perform more conventional statistics. Toxicology Branch's interpretation of the data results in the following incidences of tumors in the nasal cavities of rats fed metolachlor for 2 years:

| | DOSE (ppm) | | | |
|---|---|---|---|---|
| | 0 | 30 | 300 | 3000 |
| **Male** | | | | |
| -adenocarcinoma | 0/67 | 0/59 | 0/53 | 2/69 |
| -fibrosarcoma | 0/67 | 0/59 | 0/53 | 1/69 |
| **Female** | | | | |
| -adenocarcinoma | 0/67 | 0/58 | 0/59 | 0/69 |
| -fibrosarcoma | 0/67 | 0/58 | 0/59 | 0/69 |

Using the Cochrane-Armitage trend analysis, a value of p=0.01 (for trend) is obtained for the male treatment group. However, the more conservative Fisher's Exact Test does not yield a significant result (p=0.3).

Regarding the biological relevance of this lesion, we do not agree that the findings are completely spontaneous and unrelated to treatment. All of the references cited above, in-

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cluding that submitted by the registrant, note that true neoplasms of the nasal cavity are rare. In fact, the registrant's reference (Pour, et al., page 9) noted: "We have no reports of, nor have we observed, naturally occurring neoplasms in the upper respiratory tract". In the present study, we are faced the appearance of 2 adenocarcinomas and 1 fibrosarcoma (albeit of histogenetically distinct origins) in the same high dose male treatment group, with no apparent incidence of either tumor type in any other control or treatment group. In view of the structural similarity of this compound to alachlor (which causes a high rate of tumor formation in the nasal cavity of rats), and the absence of acceptable historical control data for this tumor type, Toxicology Branch must adopt a conservative approach and assume that these tumors are potentially related to treatment.

Therefore, we arrive at precisely the same conclusion articulated in our original review: these data are suggestive of an oncogenic response in the nasal cavities of high dose males, however the data cannot be considered as conclusive evidence. The comments submitted by the Registrant offer nothing new to consider, however the results of examinations in the low and mid dose groups do provide additional evidence for a treatment-related effect.

In conclusion, our original interpretation is unchanged, as clearly the findings in the nasal turbinate do add to the weight of evidence. The significance of these findings, if any, in relation to the carcinogenicity classification for metolachlor (currently category C) will be determined by the Toxicology Branch Peer Review Committee.

2. Regarding the significance of testicular atrophy noted in males that died on test, the registrant has supplied historical control data on the incidence of this lesion in rats examined at final sacrifice and for rats that died on test.

The submitted data demonstrate that the historical incidence of testicular atrophy (4 studies) in rats that died on test ranged from 22% to 41%. The incidence of this finding in the present study ranged from 0% in control to about 39% in the high dose group. The incidences observed in the metolachlor study are tabulated below:

| | DOSE (ppm) | | | |
|---|---|---|---|---|
| | 0 | 30 | 300 | 3000 |
| -Died on test | 0/27 | 5/26 | 7/35 | 10/26 |
| (%) | (0) | (19.2) | (20.0) | (38.5) |
| -Final sacrifice | 6/33 | 1/34 | 3/25 | 2/34 |
| (%) | (18.2) | (2.9) | (12.0) | (5.9) |

Therefore, in the opinion of the present reviewer the findings are spontaneous and not evidence of metolachlor toxicity in view of the historical incidence of this lesion and the lack of confirmatory evidence at final sacrifice. The only other effect noted in this study (other than tumors) was a decrease in weight gain at the high dose (3000 ppm), and accordingly the NOEL is now established at 300 ppm.

3. Regarding dose-selection in the mutagenicity studies, the registrant has submitted range-finding data demonstrating that the selection of doses or concentrations tested are adequate. Therefore, these studies - the chinese hamster micronucleus test (study # 831499), the DNA repair test in hepatocytes (study # 831497)- are all re-classified as Accepable Data.

The registrant also questioned our concern as to whether the test material had reached the target site in chinese hamster bone marrow study. The rodent bone marrow assay is frequently chosen for examination of clastogenic effects because a population of rapidly dividing cells is readily provided for examination. To properly assess the clastogenic potential of a chemical using this type of study, the chemical tested must be able to reach the target site, i.e. bone marrow. This is necessary to distinguish between a "no test" (lack of absorption) and a "negative test" (absorption but negative results).

It is noteworthy to indicate that the Agency has recently endorsed the concept of a limit dose of 5 g/kg for this type of assay.

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

AUG 23 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Metolachlor.
Caswell No. 188D

FROM: Reto Engler, Ph.D.
Chief, Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Richard F. Mountfort, Product Manager #23
Registration Division (TS-767)

On May 30, 1985, the Toxicology Branch Peer Review Committee, Dr. Richard Hill (OPTS, Science Advisor), and Dr. Harry Milman (Oncology Branch, OTS) met to discuss and evaluate the data base on Metolachlor, with particular reference to the oncogenic potential of the chemical.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated).

Theodore M. Farber, Ph.D. _____

Richard Hill, M.D. _____

Harry Milman, Ph.D. _____

Louis Kasza, D.V.M.,Ph.D. _____

Herbert Lacayo, Ph.D. _____

John A. Quest, Ph.D. _____

2. Reviews: (Non-panel members responsible for presentation of data; signatures indicate technical accuracy of panel report.)

Gary Burin, M.P.H. _____

D. Steven Saunders, Ph.D. _____

Laurence D. Chitlik, D.A.B.T. _____

B. Material Reviewed:

The material available for review consisted of DER's of rat and mouse chronic oncogenicity bioassays, laboratory audit reports and related memoranda on the bioassay, risk assessment information, data on other toxicology studies (mutagenicity, subchronic, and reproduction/teratology tests), and a listing of one-liners on the Metolachlor data base. A copy of the material reviewed is appended to this panel report.

C. Overview of Toxicology Issues:

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.

The initial rat chronic bioassay was performed at Industrial Biotest Laboratories (IBT) and was classified as "supplementary" data. A repeat rat chronic bioassay was performed at Hazelton-Raltech, Inc., and was classified as "CORE minimum" data. Although both studies were given careful consideration, the Peer Review Committee focused attention primarily on data in the Hazelton-Raltech rat study because of the "CORE minimum" classification.

The registrant also evaluated the oncogenic potential of Metolachlor in two mouse oncogenic feeding studies. One of the studies was performed at IBT, and a repeat study was performed at Hazelton-Raltech, Inc. Both mouse studies were classified as "CORE minimum" data, and no evidence for oncogenicity was found in either mouse study.

D. Evaluation of the Evidence:

1. Rat Chronic Studies

a) Initial IBT Study: 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. 35:3214-3223, 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.

| 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".

2. Mouse Chronic Studies:

a) Initial IBT Study: A 2-year mouse study (No. 622-07925) using dietary levels of metolachlor of 0, 30, 1000 and 3000 ppm was reviewed and classified as "CORE minimum." No oncogenic effects were noted. The Review Committee did not raise any issues concerning the results of this bioassay.

b) Repeat Hazelton-Raltech, Inc. Study: A 2-year mouse study (No. 79020) using dietary levels of Metolachlor of 0, 300, 1000 and 3000 ppm was reviewed and classified as "CORE minimum." No oncogenic effects were noted. The highest dose level tested produced a reduction in weight gain ($P < 0.05$) in male and female mice indicating that the MTD was approximated. A slight reduction in survival was also noted in high dose females (34.6% survival to end of test vs. 53.8% controls, 38.5% low dose and 46.2% mid dose) which might have been related to Sendai virus infections early in the study. The review committee had no issues concerning the results of this bioassay.

3. Mutagenicity Assays

Two genotoxicity assays were performed with Metolachlor, a mouse dominant lethal study and a Ames mutagenicity assay. Both tests were acceptable and Metolachlor was not mutagenic in either study.

4. Teratology and Reproduction Studies:

Four pivotal studies were briefly considered by the Committee: 1) Metolachlor was not teratogenic, fetotoxic, or maternally toxic at the highest dose tested (360 mg/kg/day) in a teratology study in rats (Ciba Giegy, CORE minimum); 2) Metolachlor was not teratogenic or fetotoxic at the highest dose tested (360 mg/kg/day) in rabbits, but produced maternal toxicity (lacrimation, miosis, decreased food consumption, and reduced day 12 and 18 body weights) at this dose level (Argus, CORE minimum); 3) in a 2-generation study in rats, the highest dose level (1000 ppm) reduced pup weights and parental food consumption (Toxigenics, CORE guideline); and 4) in a 3-generation study in rats the highest dose level tested (1000 ppm) was a NOEL (IBT, supplementary).

D. Weight of Evidence Considerations:

The Committee considered the following facts regarding toxicology data on Metolachlor to be of importance in a weight of the evidence determination of oncogenic potential.

1. Metolachlor was associated with a significantly elevated incidence of proliferative liver lesions (neoplastic nodules plus carcinomas combined) at the highest dose level tested (3000 ppm in the diet) only in female rats. A non significant trend of increasing neoplasic nodules was observed in male rats in both rat studies. No proliferative lesions were noted in female or male mice.

2. The significantly elevated incidence of proliferative liver lesions observed in high dose female rats (i.e., 7/60) was primarily due to the occurrence of neoplastic nodules (i.e., 6/60) rather than to hepatocellular carcinomas *per se* (i.e., 1/60). There was no apparent difference in the time-to-occurrence of the proliferative lesions (i.e., almost all liver tumors were observed at terminal sacrifice).

3. Historical control data on the proliferative liver lesions in the same strain of rat (at Hazelton-Raltech, Inc.) is available from one other study in which two control groups were employed. The incidence of these proliferative lesions were 0/46 and 1/46 for females of the two control groups.

4. The hepatic proliferative responses observed in female rats appear to be repeatable, since a similarly elevated incidence of neoplastic nodules was observed in female rats at the same dose level tested (i.e., 3000 ppm) in both the initial IBT study and the subsequent Hazelton-Raltech, Inc. study. However, as noted above, a statistically significant oncogenic response was not produced in male rats in either study at the same maximum dose level.

5. Metolachlor was not mutagenic in two genotoxicity studies performed on the compound (mouse dominant lethal study and Ames mutagenicity assay) nor were teratogenic, fetotoxic or adverse reproductive effects observed in studies in rats or rabbits.

6. Preliminary 90-day animal toxicity studies indicated that a dose of 3000 ppm did not exceed a "maximum tolerated dose" (MTD) in those subchronic tests. However, in the chronic rat bioassay, the dose of 3000 ppm appeared to approximate the MTD level as it did not adversly affect the survival of the animals but was associated with: (a) weight loss in female rats ($P < 0.05$) from study weeks 2 to 104 (which was found to be reversible following discontinuation of compound administration for one month in a satellite group of female rats after 12 months on test); (b) reduced food consumption in female rats ($P < 0.05$) at intermittent intervals throughout the study; (c) testicular atrophy with degeneration of tubular epithelium in male rats upon histological examination (the severity of the effect was similar in all treated groups but the time of onset was sooner in all groups of treated males); and (d) an increased incidence of eosinophilic foci in the livers of both male (10/59 control, 15/59 low, 14/60 mid, 21/60 high) and female (4/60 control, 7/60 low, 5/60 mid, 23/60 high) rats upon histological examination.

7. Metolachlor bears a structural resemblance to Alachlor but differs from Alachlor in toxicity and in some physical properties. Available metabolism data indicates that both Metolachlor and Alachlor are metabolized to analine derivatives. However, adequate data is not available for Metalachlor. Nonetheless, types of oncogenic responses produced by Metolachlor (proliferative liver lesions) and Alachlor (nasal turbinate, stomach and thyroid tumors) in rats are different.

E. Classification of Oncogenic Potential:

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).

Addendum:

Dr. Saunders provided the following additional information to this report on 7/30/85: (1) Data for histopathologic examination of nasal tubinates, from control and high dose male and female rats in the chronic feeding study, were recently submitted and are currently under review; (2) An *in vivo* cytogenetics study and two *in vitro* DNA repair studies were recently submitted and are currently under review.

OPP:HED:TOXJ.QUEST/R.ENGLER:sb 6/10/85 X77490 #1-D48
rew:7/17/85

CASWELL FILE

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

004725

OCT 21 1985

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.
Toxicologist, Section V
TOX/HED (TS-769)

DSS 10-10-85

THRU: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769)
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-679)

W. Tessler for L. Chitlik 10-11-85

## 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.

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.

2

# 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)

L.K.

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]**

| | Male | | Female | |
|---|---|---|---|---|
| Group | 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.

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

Table 2 [a]

INCIDENCE SUMMARY

PRINTED: 30-APR-8
PAGE: 1

STUDY NUMBER: 80030A

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=B,N,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.

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

Table 2 (Continued)[a]



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 |
| ** 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

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

DEC 14 1983

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

TO: R. Mountfort (PM#23)
Registration Division (TS-767C)
and
J. McCann, Chief
Lab Audit Program, BFSD (TS-768)

THRU: William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

SUBJECT: Review of Chronic Rat Study of Metolachlor
Accession Nos.: 250369-250375 CASWELL#188DD

Registrant: Ciba-Geigy Corp.
Agricultural Division
Greensboro, N.C.

Recommendation: It is recommended that this study be core classified as Supplementary Data. The NOEL is 30 ppm, based on atrophy of the testes with degeneration of the tubular epithelium in the mid and high dose groups. An increase in primary liver tumors is observed in the male and female high dose groups. A risk assessment may eventually be required based on this study; however, it is first recommended that a laboratory audit be conducted on this study. This is triggered by conflicting reports of the incidence of liver tumors emanating from a preliminary report and the Final Report of the study. Depending upon the results of the audit, this study may be upgraded to Core Minimum Data.

Review of Data:

Chronic Feeding, Rats. Conducted by Hazelton Raltech, Inc., Madison, WI, Study No. 80030 and submitted by Ciba-Geigy, May 2, 1983.

CD-Crl:CD(SD)BR rats were obtained from Charles River Breeding Laboratories and were acclimated for two weeks prior to testing. Seventy rats per sex were assigned to groups which were to receive either 0 or 3000 ppm. Sixty rats per sex were assigned to groups which would receive either 30 or 300 ppm. Test diet was offered ad libitum for 104 weeks of testing and was formulated with metolachlor technical. Water was available ad libitum.

All animals were individually housed in a room with a temperature of 72° ± 3 and 30 to 70% relative humidity.

Animals were examined twice daily within their cages. Once per week animals were removed and carefully examined. Starting at week 14, animals were palpated weekly for tissue masses.

Body weights were recorded weekly from weeks 0-13 and biweekly after week 16. Food consumption was recorded weekly for weeks 0-13 and biweekly after week 16 for 10 animals per group. In addition, food consumption was recorded for all animals in all groups at weeks 40, 52, 66, 78, 92 and 104.

Clinical studies were conducted on eight animals per group after 3, 6, 12, 18 and 24 months on test. At the 18th month of testing, an additional 10 animals per group were selected. Hematology consisted of RBC, WBC and leucocyte counts, hematocrit, hemoglobin and platelet counts. Clinical chemistry consisted of LDH, AST, ALT, AP, BUN, glucose, total protein, total cholesterol, direct and total bilirubin, Ca and K. Urinalysis consisted of "Ames multistix", specific gravity and microscopic examination.

All animals on test were necropsied. A total of 31 organs had tissues taken and all gross lesions and tissue masses were preserved. The following tissues were examined as reported by the registrant:

Adrenal glands
Bone marrow section (fumur)
Brain (cerebrum, cerebellum, and pons)
Cecum
Colon
Esophagus
Eyes
Gonads
Heart
Kidneys
Liver (at least two lobes)
Lungs (two coronal sections including all lobes and mainstem bronchii)
Lymph nodes (cervical and mesenteric)
Mammary gland
Muscle (skeletal)
Urinary bladders
Uterus
Optic nerve
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

All animals on test had tissues microscopically examined. The following organs were weighed prior to fixation: heart, liver, spleen, kidney, gonads and brain.

2

Ten males and 10 females from the control and high dose groups were randomly selected after 12 months for a recovery study. Five of these animals were sacrificed immediately and 5 were placed on control diet (absent test compound) for 4 weeks. Clinical studies, organ weight determinations, gross and histopathology determinations for recovery animals were identical to those that continued on test. Statistical comparisons were conducted by the registrant on all parameters (this reviewer independently conducted statistical analysis for the liver tumor incidences using the Fisher's Exact Test).

Results:

Diet analyses were conducted for all dose levels at weeks 1-4 and for randomly selected test diets on a weekly basis for the remainder of the test period. No metolachlor (< 5.0 ppm) was found in control diets, time-weighted averages of 29.1, 273, and 2851 ppm were found in the diet.

Survival over the course of the study was adequate with 54, 57, 42 and 57% of the control, 30, 300 and 3000 ppm dose groups surviving until study termination at 24 months. It did not appear that the survival rate was influenced by test compound administration.

At week 9, animals in all groups began to show clinical symptoms indicative of sialodacryoadenitis virus. These symptoms included "palpable enlargement of the submaxillary salivary glands, a generalized edema in the cervical and mandibular areas, and red-tinged (porphyrin) discharges in the nasal and ocular areas."

The symptoms persisted for only 2-3 days and animals showed no further indication of disease. In addition to the above described clinical signs, animals lost weight (approximately 5 grams) during the time of infection. No animals died during this period. The disease outbreak is considered by this reviewer to be of little consequence to the interpretation of the study.

Mean body weights of females in the high dose group were consistently less than controls from week 2 until study termination. For 26 of the 59 intervals at which animals were weighed, this difference was significant at the $p < .01$ level. Neither male body weights nor low or mid dose female dose groups were affected by treatment. Food consumption in high dose females was slightly less than controls and the difference was statistically significant ($p < .05$) at 10 of 59 intervals with seven of these intervals between weeks 5 and 18. Male food consumption appeared unaffected by treatment.

Organ weights and organ to body weight ratios were similar among all dose groups.

A variety of differences in the clinical pathology measurements were found between control and dosed groups at various intervals but no consistent dose-related effects were apparent with one exception. Aspartate aminotransferase activity was less than controls in both sexes at 3000 ppm at 12 months and the decrease was significant ($p < .01$) in males. Nonstatistically significant decreases in AST activity were noted at 3000 ppm; at other intervals for both sexes and in females at the 300 ppm dose level at 18 and 24 months. It should be noted that the recovery study found that AST values in high group, which were depressed at 12 months, increased after one month recovery period to a level that was not statistically significant. The recovery study also suggests that body weight depression in the 3000 ppm dose level also is reversible with most of the difference between control and high dose body weights disappearing through the one month recovery period.

Gross pathology findings of the scheduled sacrifice, moribund sacrifice and "died on test" animals were unremarkable.

The incidence of neoplastic nodules and hepatocellular carcinomas reported in the Final Report was as follows:

**Males**

| | Dose | | | |
|---|---|---|---|---|
| | 0 ppm | 30 ppm | 300 ppm | 3000 ppm |
| Neoplastic nodules | 0 | 0 | 0 | 4 |
| Hepatocellular Carcinomas | 2 | 1 | 3 | 2 |
| Total Examined | 59 | 59 | 60 | 60 |

**Females**

| | Dose | | | |
|---|---|---|---|---|
| | 0 ppm | 30 ppm | 300 ppm | 3000 ppm |
| Neoplastic Nodules | 0 | 0 | 1 | 4 |
| Hepatocellular Carcinomas | 0 | 0 | 0 | 2 |
| Total Examined | 60 | 60 | 60 | 60 |
| Total Examined After the Observation of the First Animal with Tumor | 45 | 43 | 42 | 50 |

The numbers of animals examined after the observation of the first of females dying with tumors (a high dose animal observed at week 90) were 45, 43, 42 and 50 for the control, 30, 300 and 3000 ppm dose level females, respectively. Although the registrant asserts that the incidence of these tumors in high dose females is not statistically significant compared to the control group, this reviewer found statistical significance with p = .0183 (Fisher's Exact test, 0/45 vs. 6/50 for the control vs. high dose groups).

The incidence of these tumors in female rats at this laboratory can only be assessed from a single other study as indicated on p. 36 of Vol. 1 of the registrants submission. Apparently two control groups were used in the historical study and the incidence of these tumors were 0/47 and 1/46 for females of the two groups.

The incidence of other tumor types was unremarkable and did not appear to be related to treatment.

It should be noted that the increased incidence of these tumors is consistent with IBT Study No. 622-07926, conducted with the same doses and classified as "Supplementary Data". It should also be noted that a letter from the registrants dated December 9, 1983 (Attachment A) reported a different incidence of liver tumors in this study than was subsequently reported by the registrants in the Final Report. The incidence of liver tumors originally reported as 2, 2, 2 and 9 for control, low, mid and high dose males and 0, 1, 2, and 7 for control, low, mid and high dose females. This reviewer has requested an explanation for the differing incidences of primary liver tumors in the two reports of the the same study and the response from the registrant was received on November 2, 1983 (Attachment B). The response states that "Subsequently, liver sections were reviewed during the examination of all other protocol tissues and it became apparent that some of the "original diagnoses" would have to be changed. Primarily this was because the presence or absence of "compression of surrounding parenchyma" had not been given uniform consideration during the original examination...The primary difference in the two sets of data was that some of the lesions originally classified as proliferative foci (neoplastic nodules) were ultimately classified as foci of cellular change due to a lack of compression of surrounding parenchyma."

Microscopically, atrophy of the testes with degeneration of the tubular epithelium was found to a greater extent in mid and high dose animals than in the control group, with 6/60, 6/60, 10/60 and 12/60 animals affected in the control, low, mid, and high dose groups, respectively. Although the severity of this finding appeared similar in all groups the time of observation of the atrophy was sooner in the treated groups, with 0/27, 5/26, 7/35 and 10/26 of those animals that died-on-test animals having this finding. An increasd incidence of eosinophilic foci were observed in the livers of high dose males and females with 10/59, 15/59, 14/60, 21/60 (males) and 4/60, 7/60, 5/60 and 23/60 (females) affected in the control, low, mid and high dose groups, respectively. Other pathological findings are considered by this reviewer to be incidental to test compound administration.

<u>Classification</u>: Supplementary Data.

The NOEL for non-neoplastic effects is 30 ppm based on testicular atrophy with degeneration of the tubular epithelium. An increased incidence of neoplastic nodules/hepatocellular carcinomas were observed in this study. Due to a difference in the incidence of liver tumor reported in a preliminary report and the Final Report of the study, the conduct of a laboratory audit is recommended.

Gary J. Burin, Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

12/14/83

## ONCOGENICITY

### Summary

At this time oncogenicity data on technical Metolachlor is limited to the study done by Industrial Bio-Test Laboratories, Inc., Report No. 622-07925, December 15, 1977:

| Species | Dose levels & Conclusion |
|---|---|
| Charles River CD-1 Albino Mice | Not oncogenic when fed at dietary levels of 0, 30, 1000 and 3000 ppm for 18 months to males and 20 months to females. |

The study, as submitted, has a number of shortcomings. Essentially these relate to poor reporting of (1) actual test procedure, (2) actual observations, and (3) unexpected problems which developed during the conduct of this study. This study report is misleading as well as incomplete and must be revised to accurately reflect the conduct of this study.

The histopathology data, on the other hand, appears complete and has been validated by Ceiba-Geigy. This histopathology data revealed that Metolachlor did not induce an increase in neoplastic or non-neoplastic lesions when fed to Charles River CD-1 mice at levels of 0, 30, 1000 and 3000 ppm.

There are indications that animal husbandry was far from ideal during the conduct of this study and that this contributed to the reduced longevity and body weights of these mice (e.g. - males were sacrificed several months earlier than females to ensure adequate numbers for examination).

The study was audited by Ceiba-Geigy and stated to be valid. It appears that their primary concern in this audit was the pathology data, but their attention should have been given to the entire conduct of the study.

If and/or when the revised report is submitted reevaluation may be necessary, but at this time the pathology data supports the report conclusions regarding oncogenicity.

Laurence D. Chitlik

1. CHEMICAL: Metolachlor (CGA24705)

2. FORMULATION: CGA-24705 Technical (FL-750227 99.9% active and FL-752105 96.5% active)

3. CITATION: Gesme, J.; Albanese E.,; Marias, A.J.; Arces, R.J. (December 15, 1977) Carcinogenicity Study with CGA-24705 Technical in Albino Mice: IBT No. 622-07925 (8532-07925). Received January 18, 1978 under 7F1913. (Unpublished report prepared by Industrial Bio-Test Laboratories, Inc. for CIBA-GEIGY Corp., Greensboro, N.C.: CDL: 096719

4. TRADE SECRET CLAIM: Yes

5. REASON FOR REVIEW: Generic Standard for Metolachlor

6. REVIEWED BY: Laurence D. Chitlik
Toxicologist, Toxicology Branch
Registration Division

7. DATE OF REVIEW: March 1, 1978

8. TEST TYPE: Oncogenicity

A. Materials and Methods: Four hundred (200 males and 200 females) Charles River CD-1 Albino mice aged 35-40 days were received, observed for an additional 7 day period and then assigned to 4 groups as follows:

| Group | Dietary level in PPM | Number of Animals Males | Females |
|---|---|---|---|
| Control | 0 | 50 | 50 |
| T-I | 30 | 50 | 50 |
| T-II | 1,000 | 50 | 50 |
| T-III | 3,000 | 50 | 50 |

Males were housed in individual cages while females were housed 5 to a cage. Cages were identified by color-coded cards identifying the project number, dietary level, animal number and sex. Individual animals were identified with ear tags. Observations for toxic signs and/or death were conducted twice

daily. Necropsies were conducted on all animals found dead (unless autolyzed), sacrificed in extremis, and sacrificed at term. Tissues were fixed in 10% neutral buffered formalin. The following tissues were prepared for microscopic examinations (stained with Hematoxylin-Eosin) and examined (from all animals, except autolyzed):

Adrenal Glands
Aorta (thoracic segment)
Brain (cerebrum, cerebellum, pons)
Caecum
Colon
Epididymis
Esophagus
Eyes with optic nerve
Gonads
Heart
Kidneys
Liver
Lung
Lymph Nodes (cervical, mesenteric)

Mammary Gland
Muscle (skeletal)
ALL NEOPLASMS & SUSPECT NEOPLASMS

Pancreas
Parathyroid Gland
Peripheral Nerve
Pituitary Gland
Prostate Gland
Salivary Gland (submaxillary, sublingual, parotid)
Small Intestine (duodenum, jejunum,
Spinal Cord
Spleen
Sternum with marrow
Stomach (cardia, fundus, pyloris)
Thyroid Glands
Trachea
Urinary bladder
Uterus

Body weight data were requested as an addendum to the protocol after the study was in progress. The first body weights were taken at month 5 and then monthly thereafter.

The test material was mixed with Purina Rat Chow in a high-speed Hobart blender. Test material batch FL-750227 99.9% active ingredient was used during the first 33 weeks while test material batch FL-752105 96.5% A.I. was used for the remainder of the study. The diets were to be corrected for the change in active ingredient. Fresh diets were prepared weekly. Water and diet were to be available ad libitum and daily checks were made to ensure this.

Body weight data were statistically analyzed by using a one-way analysis of variance. Any significant body weight effects were then analyzed by either

the Tukey's (equal population size) or the Scheffe's (unequal population size) Multiple Comparison Test. Historical data for mice of this age and strain were also used for the final interpretation of results.

B. REPORTED RESULTS

Average body weights among animals fed 3,000 ppm were slightly lower throughout most of the study as compared to controls. At 1,000 ppm or lower dose levels, body weights were considered comparable to controls, although "meaningful evaluation" could not be determined since body weights were not available during the first 4 months of the study.

No "treatment-related mortalities" were evident, but males were sacrificed at 18 months and females after 20 months to ensure an adequate number of animals at final sacrifice. No unusual behavorial reactions were observed during the study.

Microscopic examinations were conducted by R.J. Arceo, M.D., Staff Pathologist. He concluded that there were no treatment-related morphologic changes. Furthermore, lesions were of a natural origin and occurred with a comparable incidence and relative severity among both control and test animals. The incidence, site of origin and classification of neoplasms compared favorably with historical IBT data for mice of this age and strain. The pathology report indicated that missing tissues (including some thyroids, parathyroids and pituitary) occurred evenly among the test groups and did not interfere with conclusions reached. Some tissues not called for in the protocol (aorta, esophagus, epididymides, seminal vesicles and lacrimal gland) were processed for some animals.

C. DISCUSSION

A number of questions arose during the review of this study and Ciba-Geigy was first contacted 2/8/78, in an effort to resolve them. Other calls followed as new questions developed and Ciba-Geigy submitted

a number of addendums to the study including raw data for observations, and a mouse 28-day range finding study not previously submitted (as well as its audit report).

Originally, one question existed concerning very significant body weight losses in males (especially) and females at month 11 and continuing through month 13. (See GRAPH attached.) The report stated that "No unusual behavioral reactions were observed during the investigation" and also that "Meaningful evaluation of the body weight gain cannot be determined since no body weights were collected during the first four months of the testing." Also, the report did not indicate the strain of mice tested, which was later stated to be Charles River CD-1.

At first Ciba-Geigy (Dr. Rolofson and Dr. Sumner) checked with IBT and could gain no explanation for the weight loss. It was then requested that daily observations data be submitted to Registration Division to determine whether some toxic signs, possible overlooked by IBT, might help explain the weight loss and also demonstrate that dose levels were at an adequate level (Note: The report indicated that the only compound effects were slightly lower average body weights at 3,000 ppm, but also at the same time meaningful evaluation was not possible!).

Ciba-Geigy submitted an addendum to the report dated February 16, 1978. It contained a mouse 28-day range finding study IBT No. 622-07857 (not previously submitted) demonstrating dose levels in this oncogenicity study to be adequate and at or near the MTD. At 10,000 ppm, in this range finding study, moderate weight reduction occurred and no toxic signs were reported.

The addendum also included some discussion by Dr. Darrell Sumner (of Ciba-Geigy) of observations not previously noted in the IBT report. Dr. Sumner stated that no observations were recorded until the 5th month and that dermal lesions were noted at the

eighth month. He also indicated that through much of the study observations were recorded every two weeks rather than twice daily as the test report indicated. It was also explained that allopecia and "eye and ear infections" were common to all groups and tremors, paralysis, distended abdomens, and diarrhea were noted in all groups near the end of the study. He then went on to discuss the weight losses and their significance and commented that the compound does not elicit easily observed toxic signs...

The submission also included a Ciba-Geigy audit report on this study (CGA-24705 range finding study, IBT No. 622-07857, November 21, 1975, dated February 15, 1978).

The discrepancies noted between the report indicating observations were made twice daily versus Ciba-Geigy indicating that they were not made for the first 5 months and after that usually twice per month (but sometimes monthly or sometimes once per week), prompted request of the raw data for the daily observations (received March 9, 1978).

The raw data were reviewed and among other things, the following was noted:

1. At least 10 animals demonstrated dermal lesions as early as July 26, 1976. All of these animals were sacrificed on August 17, 1976 and August 19, 1976 "in extremis."

2. At least 20 animals, 8 T-III males and 9 T-II females exhibited allopecia. At least 6 control animals also demonstrated allopecia.

3. At least 25 test animals and a number of control animals demonstrated "eye irritation."

4. A fair number of test and control animals exhibited equilibrium problems and blood in ear canal.

Several telephone conversations with Dr. Sumner followed review of this data, 3/10/78, and 3/13/78, and an additional submission was received from Ciba-Geigy, 3/15/78 (7 pages).

Mr. Chitlik pointed out to Dr. Sumner that discussion relating to these observations should have been included in the IBT oncogenicity study whether or not IBT toxicologists thought they were compound related. Such determinations have to be made by EPA toxicologists and no mention of any of these or other observations were included in the report submitted to Registration Division.

Mr. Chitlik suggested that the allopecia and "dermal lesions" noted in the study may be related to a mite infestation and inquired as to whether a veterinarian or other IBT staff had made an effort to determine the cause of these observations as well as the eye and ear irritation noted in many animals. In response, the Ciba-Geigy submission of 3/15/78, which includes a memo to Dr. Sumner as well as a number of in-house memos discussing the "daily" observations as well as possible reasons for the body weight losses in both control and test animals.

The memo of A.J. Marias, 3/14/78, indicated the following:

1. The glass jars were replaced by stainless stell feeders in November and December of 1976. Note: This coincides with body weight losses in this study. There was no mention of this in the test report.

2. The feeders did not function properly and had to be modified to allow a greater amount of diet to fill the trough at the bottom of the feeder. Note: There was no mention of this in the test report.

3. The diet was changed during the study from Purina Mouse Chow to Purina Rat Chow. The Purina Rat Chow had a five percent lower fat content. Note: The test report did not indicate a change in diet. It indicates animals were fed Purina Rat Chow only. No date of this change has been indicated by IBT.

4. Marias also stated that during "Animal care meetings" a skin lesion problem, "associated with a number of mouse studies" was discussed and the cause was not readily determined. Dr. Robl was stated to have been unable to observe mites upon microscopic examination on numerous skin scrapings (from this study?) but that mice on related studies revealed the presence of occasional mites associated with the dermatitis.

5. The affected animals were removed from the study and sacrificed in order to avoid spread of the lesion.

   Note: Eleven mice appear to have been sacrificed in the study related to this (Control males 3, 29, 33, 48; T-I male 102; T-II males 222, 226, 227, 230, 247; T-III male 343). They were sacrificed "in extremis" on 8/17/76 and 8/18/76 according to the observations raw data. They are identified as moribunds in the report, yet they were NOT moribund, they were sacrificed to control this problem. The report does not discuss this sacrifice and obviously no discussion of the associated problem is included either.

The memo of A.J. Marias referenced a memo of Dr. Robl (8/18/76). From this memo, the following was determined:

1. Room 9 contained 6 other mouse oncogenic studies, possibly on 6 other compounds.

   Note: The possibility for cross contamination of diet due to such practices is greatly increased. Tremors were noted in all females of groups II and

III on 1/17/77 from 9-10 a.m. This was not noted as occurring at any other time during the study and has so far been unexplained by Ciga-Geigy or IBT. This practice is totally unacceptable according to the proposed GLP's. It may be prudent to determine chemical nature of other compounds run in that room.

2. One study (unidentified) within this room must have had a severe problem and approximately 10 animals in each of the other studies had contracted it... Those 10 animals per study were to be sacrificed.

3. The animals within this study, as well as the others within this room, were to be "rotated into properly cleaned cages as soon as possible." Obviously the animal husbandry was poor.

4. Dr. Robl did not have tissues processed from this other study to determine the cause. He stated that, "Whatever the cause of this problem was, evidently it has been controlled through sacrifice of affected animals."

This reviewer does not believe that if this was a mite infestation, it was controlled by sacrificing these 11 animals. The fairly widespread eye and ear irritation and allopecia which persisted through the study especially in the male animals, is still not completely understood. Possibly the associated dietary and other problems (i.e. difficulties with new feeders) discussed are related to the reduced longevity in this study.

D. CONCLUSIONS

Nearly the entire discussion section of this report relates to poor reporting of data and poor animal husbandry. Some of the findings noted are certainly related to the reduced longevity in this study. These sections of the report should be rewritten by IBT to more accurately reflect the findings in the study.

Review of the histopathology data presented in this study revealed that Metolachlor did not induce any treatment related changes when fed at 0, 30, 1,000, or 3,000 ppm. No increase in either neoplastic or non-neoplastic lesions was noted. A mouse range finding study was conducted which indicated 3,000 ppm at least approaches the MTD even though body weight data and observations within this carcinogenicity study are of little or no use (see DISCUSSION section).

Even with the shortcoming s of this study, it is concluded that Metolachlor is not carcinogenic to Charles River CD-1 mice when fed at levels of 30, 1,000 and 3,000 ppm.

This report was audited by D.D. Sumner and R.H. Ross, Jr. of Ciba-Geigy, 1/12/78. An addendum to this audit was submitted 2/16/78. They concluded the study was valid after review of the raw data. Quite a number of deficiencies have been noted in the Ciba-Geigy audit. Nearly all of the findings mentioned in the discussion section of this review were originally omitted from their audit report.

Laurence D Chitlik

Laurence Chitlik
Toxicology Branch

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

8-3-84

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metolachlor Mouse Oncogenicity Study; EPA Reg. No. 100-587; Accession Nos. 248722-25; CASWELL #188DD

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

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

D. Stephen Saunders Jr.
7-30-84

THRU: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769)
and
William L. Burnam
Chief, Toxicology Branch
HED (TS-769)

LDC 7/30/84
WLB 8/2/84

## Action Requested

Review of metolachlor mouse oncogenicity study.

## Recommendations

This study has been classified as Core-Minimum data. The following minor points are noted:

1) The study report states that the technical material was used in the study, and that the test compound was analyzed by the registrant at study initiation and every three months thereafter. These data "are on file with Ciba-Geigy", and should be provided to the Agency.

2) The method of sacrifice of test animals was not described in the study report.

If these minor points are clarified, the study can be upgraded to Core-Guideline.

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 the 3000 ppm dose was a Maximally Tolerated Dose (MTD). No other significant chronic effects were noted in this study (see review).

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

23/37 time points measured after this time statistically significant 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 ne  sy were compared to microscopic findings and tabulated by the investigators.  number of gross findings at necropsy, principally in the liver, kidney and  ph 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 | | | Dose (ppm) | Females | | | |
|---|---|---|---|---|---|---|---|---|
| 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 2. STATISTICS

DATE: JULY 11,1984

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

| 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

TITLE: METOLACHLOR-FEMALES

DATE: JULY 10,198

REMARKS: TOT. ALV. CARCENOMA = 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. CARCENOMA = 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. CARCENOMA = 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=DOT+MS=+IS+TS=ALL ALV. CARCENOMA

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=DOT+MS=+IS+TS=ALL ALV. CARCENOMA

| 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=DOT+MS=+IS+TS=ALL ALV. CARCENOMA

| 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

Metolachlor

Input Program

```
ct fisher def
1
//LHEX2CL JOB (WMJ1,352,A),LACAYO
/*CNTL NEG1DZT,SHR
// EXEC FORVLKGO,LIBDISK=FILE02,
// LIBNAME='NEG1DZT.STAT.LOAD'
//LOAD.SYSLIN DD *
  INCLUDE SYSLIB(C2X2)
  ENTRY MAIN
//GO.FT01F001 DD *
1 -1 1
.025 .025
-1
METOLACHLOR-MALES,CONTROL VS ALL DOSES AT 18 MO
0,8 11,24
-1
METO.-MALES,CONTROL VS ALL DOSES AT 24 MO.
5,20 26,84
-1
METO.-FEM,CONTROL VS ALL DOSES AT 18 MO
2,8 8,24
//
```

Meto-Fem, Contr vs all doses at 24 mo.

6,28 1 23,61

```
1
-                                                    DIAGNOSTIC MESSAGE DIRE
      IEW0201 WARNING - OVERLAY STRUCTURE CONTAINS ONLY ONE SEGMENT --
OPTION CANCELED.

  1
  0IFY215I VCVTH - ILLEGAL DECIMAL CHARACTER (O)
  0**** START OF BUFFER CONTENTS ****
   0,8 11,24
  0**** END OF BUFFER CONTENTS ****
  0TRACEBACK 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

   ***  WARNING  LOWER LIMIT MUST BE 0;
```

16.

*** SINGLE/COMBINED 2X2 TABLE PROGRAM JAN/16/8 *** CPU TIME=
SEC.
1

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



EXACT CONFIDENCE LIMITS FOR PSI

SEC.
1

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

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 0.660090 < 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 0.671158 < PSI < 7.144249

*** SINGLE/COMBINED 2X2 TABLE PROGRAM JAN/16/84 *** CPU TIME=
SEC.
0 MESSAGE SUMMARY: MESSAGE NUMBER - COUNT
0 215 1

CASWELL FILE

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

JAN 29 1982

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: January 28, 1982

SUBJECT: Addendum to Metolachlor Memo of October 7, 1981; Risk Assessment for Additional New Uses (Including Potatoes, PP#9F2203) & 100-597
Tox. Chem #188DD

FROM: Gary J. Burin, Toxicologist
Toxicology Branch/HED (TS-769)

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

THRU: Orville E. Paynter, Chief
Toxicology Branch/HED (TS-769)

Background Information: The registrant, Ciba-Geigy Corporation, has requested the establishment of permanent tolerances for residues of metolachlor on sunflowers (0.3 ppm), sunflower meal and hulls (0.6 ppm), seed and pod vegetables (0.3 ppm), sweet corn and popcorn (0.1 ppm), cottonseed (0.1 ppm), flaxseed (0.2 ppm), flaw straw (0.6 ppm), flaxseed meal (0.4 ppm) flax hulls (0.4 ppm) and potatoes (0.2 ppm - received on 9/30/81). In my memo of October 7, 1981, I noted the IBT chronic rat study was positive in females for primary liver tumors and that a risk assessment would be required for these additional new uses. Comments from the registrant regarding the oncogenicity of Metolachlor were addressed in the memo of December 10, 1981 from this reviewer and Dr. Louis Kasza. A risk assessment for the requested uses has recently been completed and is discussed below.

Recommendations: A risk assessment based on the chronic rat feeding study conducted at IBT indicates that the point estimate of oncogenic risk resulting from dietary exposure to existing and proposed tolerances is $2.167 \times 10^{-7}$; The upper 95% bound on this increased risk estimate is $8.0 \times 10^{-7}$.

Additional risk estimates expressed at the upper 95% confidence level are as follows (assuming 10% dermal absorption):

| | |
|---|---|
| Mixer/Loader (Farmer) | $4.5 \times 10^{-8}$ |
| Mixer/Loader (Custom Applicator) | $6.7 \times 10^{-7}$ |
| Applicator (Farmer) | $3.3 \times 10^{-6}$ |
| Applicator (Custom Applicator) | $4.9 \times 10^{-5}$ |

(Exposure estimates are based on the memo of January 22, 1982 from Ed Moroski of Environmental Fate Branch.) Toxicology Branch defers to the Administrator regarding the acceptability of the oncogenic risks. Aside from the issue of acceptability of oncogenic risk, Toxicology Branch does not object to the aforementioned proposed tolerances. It is recommended that the oncogenic potential of Metolachlor be reassessed after the chronic rat feeding study, which is now underway, is submitted and evaluated.

Discussion: (See memos of October 7, 1981 and December 12, 1981 from this reviewer for evaluation and additional discussion of this study.) The following data was used in the estimation of oncogenic risk:

TABLE I

Primary Liver Tumors in Females from IBT No. 622-07926

| | PPM | | | | |
|---|---|---|---|---|---|
| | 0 | 30 | 300 | 1000 | 3000 |
| Hypertrophic-Hyperplastic nodule | 0 | 1 | 2 | 1 | 3 |
| Hypertrophic-Hyperplastic nodules (two or more) | 1 | 0 | 1 | 2 | 6 |
| Cholangioma | 0 | 0 | 1 | 0 | 0 |
| Cystic cholangioma | 2 | 2 | 1 | 2 | 6 |
| Hepatocellular carcinoma | 0 | 0 | 0 | 0 | 2 |
| Total (# animals with primary liver tumors) | 3 | 3 | 5 | 5 | 14* |
| Number examined (month 13-Final sacrifice) | 54 | 58 | 60 | 60 | 60 |

*Three animals each bore two primary liver tumors.

A mathematical risk assessment, was performed by Toxicology Branch statistician, Mr. Bertram Litt using a multistage (Crump), multihit (van Ryzin) model and the Mantel-Bryan procedure. The multistage model was selected because it presents a reasonable fit to the data and represent the most conservative approach i.e. a "worst case" approach. The program output is as follows:

```
TITLE :   METRAT.FEMALE/LIVER TUMORS SET #1 (MULT 9 EDOSES)

CLASS( 1) HAS   54 MEMBERS WITH    3 RESPONSES TO DOSE OF          0.0
CLASS( 2) HAS   58 MEMBERS WITH    3 RESPONSES TO DOSE OF          1.40000
CLASS( 3) HAS   60 MEMBERS WITH    5 RESPONSES TO DOSE OF         14.60000
CLASS( 4) HAS   60 MEMBERS WITH    5 RESPONSES TO DOSE OF         54.00000
CLASS( 5) HAS   60 MEMBERS WITH   14 RESPONSES TO DOSE OF        140.00000
0KOP = 1 GRADIENT MOVES = 25

THE COEFFICIENTS OF THE POLYNOMIAL OF DEGREE  4 THAT MAXIMIZES THE LIKELIHOOD OF THE DATA ARE :
                    Q( 0) = 0.60862028960-01
                    Q( 1) = 0.55570767280-03
                    Q( 2) = 0.0
                    Q( 3) = 0.0
                    Q( 4) = 0.32953291610-09
     TEST OF HYPOTHESIS: Q(1) = 0
0    PR(.TEST STATISTIC) =  0.34906241760+00
0    LIKELIHOOD RATIO  0.15042993500+00
```

==========

```
0CONFIDENCE LIMITS ON EXTRA RISK AT THE  9 ENVIRONMENTAL DOSES INPUT
0CONFIDENCE LIMITS FOR DOSE OF  0.6670000D-06  M.L.E. FOR EXTRA RISK IS  0.3706570D-09
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.1204280D-08    0.1362300D-08    0.1494340D-08    0.1648080D-08
0CONFIDENCE LIMITS FOR DOSE OF  0.8890000D-06  M.L.E. FOR EXTRA RISK IS  0.4940240D-09
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.1605110D-08    0.1815720D-08    0.1991700D-08    0.2196620D-08
0CONFIDENCE LIMITS FOR DOSE OF  0.1000000D-05  M.L.E. FOR EXTRA RISK IS  0.5557080D-09
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
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        0.1805520D-08    0.2042430D-08    0.2240390D-08    0.2470890D-08
0CONFIDENCE LIMITS FOR DOSE OF  0.1670000D-05  M.L.E. FOR EXTRA RISK IS  0.9280320D-09
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.3015220D-08    0.3410860D-08    0.3741440D-08    0.4126380D-08
0CONFIDENCE LIMITS FOR DOSE OF  0.1590000D-04  M.L.E. FOR EXTRA RISK IS  0.8835750D-08
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
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        0.2870780D-07    0.3247460D-07    0.3562210D-07    0.3928710D-07
0CONFIDENCE LIMITS FOR DOSE OF  0.1210000D-03  M.L.E. FOR EXTRA RISK IS  0.6724060D-07
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
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        0.2184680D-06    0.2471340D-06    0.2710870D-06    0.2989770D-06
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0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
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        0.2202730D-06    0.2491760D-06    0.2733270D-06    0.3014480D-06
0CONFIDENCE LIMITS FOR DOSE OF  0.1270000D-03  M.L.E. FOR EXTRA RISK IS  0.7057490D-07
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
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0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.7041530D-06    0.7965480D-06    0.8737500D-06    0.9636460D-06
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Flax Seed

Popcorn

Sunflower

Cottonseed

Sweet Corn

Potatoes

Seed and Pod Vegetables

All Existing Tolerances

Total Existing & Proposed Tolerances

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==========================================================================================
0CONFIDENCE LIMITS BASED ON THE MULTI-STAGE MODEL  ( KOP = 1 )
-CONFIDENCE LIMITS FOR A RISK OF  0.100000D+00  M.L.E. DOSE =  0.1082302496D+03
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.213866D+00     0.233767D+00     0.250009D+00     0.268488D+00
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.564954D+02     0.505337D+02     0.463490D+02     0.422171D+02
-CONFIDENCE LIMITS FOR A RISK OF  0.100000D-01  M.L.E. DOSE =  0.1802308212D+02
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.320509D-01     0.361751D-01     0.396077D-01     0.435892D-01
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.556627D+01     0.492068D+01     0.448592D+01     0.406746D+01
-CONFIDENCE LIMITS FOR A RISK OF  0.100000D-02  M.L.E. DOSE =  0.1800401398D+01
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.324539D-02     0.367045D-02     0.402547D-02     0.443871D-02
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.554134D+00     0.489858D+00     0.446575D+00     0.404915D+00
-CONFIDENCE LIMITS FOR A RISK OF  0.100000D-03  M.L.E. DOSE =  0.1799597250D+00
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.324868D-03     0.367488D-03     0.403098D-03     0.444562D-03
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.553885D-01     0.489637D-01     0.446374D-01     0.404733D-01
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0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.324901D-04     0.367532D-04     0.403153D-04     0.444631D-04
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.553860D-02     0.489615D-02     0.446354D-02     0.404715D-02
-CONFIDENCE LIMITS FOR A RISK OF  0.100000D-05  M.L.E. DOSE =  0.1799508175D-02
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.324904D-05     0.367536D-05     0.403158D-05     0.444637D-05
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.553857D-03     0.489613D-03     0.446352D-03     0.404713D-03
-CONFIDENCE LIMITS FOR A RISK OF  0.100000D-06  M.L.E. DOSE =  0.1799507365D-03
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
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        0.324905D-06     0.367537D-06     0.403159D-06     0.444638D-06
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.553857D-04     0.489613D-04     0.446352D-04     0.404713D-04
-CONFIDENCE LIMITS FOR A RISK OF  0.100000D-07  M.L.E. DOSE =  0.1799507365D-04
0       UPPER CONFIDENCE LIMITS ON EXTRA RISK:
            90%              95%              97.5%            99%
        0.324905D-07     0.367537D-07     0.403159D-07     0.444638D-07
0       LOWER CONFIDENCE LIMITS ON SAFE DOSE:
            90%              95%              97.5%            99%
        0.553857D-05     0.489613D-05     0.446352D-05     0.404713D-05
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$\therefore Q_1^* = 2.04 \times 10^{-3}$

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

OCT 7 1981

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: October 5, 1981

SUBJECT: Proposed tolerances for Metolachlor on Sunflowers (0.3 ppm), Sunflower Meal and Hulls (0.6 ppm), PP#0F2416; Seed and Pod Vegetables (0.3 ppm), PP#1F2495; Sweet Corn and Popcorn (0.1 ppm), PP#1F2521; Cottonseed (0.1 ppm), 1F2506; Flaxseed (0.2 ppm), Flax straw (0.6 ppm), Flaxseed meal (0.4 ppm) and Flax hulls (0.4 ppm), 0F2417 and 1H5293, Tox. Chem. No. 188DD, Acc. Nos. 244166, 099628, 099626, 070048

FROM: Gary J. Burin, Toxicologist
Toxicology Branch, HED (TS-769)
Gary J Burin 10/5/81

TO: Richard Mountfort (23)
Registration Division (TS-767)
10/5/81

THRU: William Burnam, Acting Chief
Toxicology Branch, HED (TS-769)
WB 10.5.81

Requested Actions: Establishment of a permanent tolerances for residues of Metolachlor on sunflowers (0.3 ppm), sunflower meal and hulls (0.6 ppm), seed and pod vegetables (0.3 ppm), sweet corn and popcorn (0.1 ppm), cottonseed (0.1 ppm), flaxseed (0.2 ppm), flax straw (0.6 ppm), flaxseed meal (0.4) and flax hulls (0.4 ppm).

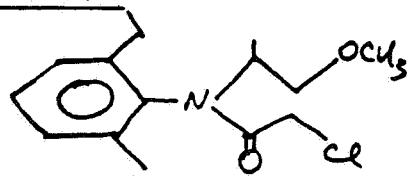
Recommendation:

Toxicology Branch is unable, at this time, to make a recommendation on the requested tolerances. Because the IBT Rat Chronic Feeding Study was positive in females at the high dose group, a risk assessment will be needed for the requested tolerances. This risk assessment is currently in progress. Toxicology Branch defers to RCB on the adequacy of existing meat and milk tolerances for the requested tolerances.

Common Names: Metolachlor, CGA-24705

Chemical Name: 2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl ethyl) acetamide

Chemical Structure:




Formulation: Dual 8E

## Background Information

Toxicology Branch noted the need for long-term feeding studies, including an oncogenic evaluation, upon review of the first request by Ciba-Geigy for permanent tolerances of Metolachlor on May 27, 1975. An 18 month mouse oncogenicity study and a 2 year rat chronic feeding/oncogenicity study were received on 2/15/78 and on 1/18/78, respectively. Both of these studies were conducted at Industrial Biotest Laboratories and thus required validation prior to use in meeting regulatory requirements (For a summary of these and other studies submitted pursuant to the registration and petition for tolerances of Metolachlor, see the memo of February 7, 1980 from L. Chitlik. Also, see Toxicology Data Summary section of this memo for a listing of these studies). On December 17, 1979, L. Anderson of Toxicology Branch determined that the aforementioned chronic rat study was "Invalid" as a chronic feeding and "Supplementary" for oncogenic evaluation. Ciba-Geigy then contracted with the consulting firm of Drill, Friess, Hays, Loomis and Shaffer, Inc. to conduct a retrospective audit of the study. Their findings were discussed in the meeting of January 27, 1981 between EPA and Ciba-Geigy. As a result of that meeting, Toxicology Branch agreed to upgrade the study to Supplementary Data as an oncogenicity and as a chronic feeding study (See memo of July 28, 1981 from G. Burin and L. Chitlik). Due to the upgrading of this study to supplementary, an evaluation of the data was then necessary and a review is included in this action. The IBT mouse study has been classified as valid (See memo of December 13, 1979 from H. Spencer).

## Toxicology Data Summary

| Study | Validity and/or Core Classification | Results |
|---|---|---|
| 2-year rat chronic study with oncogenicity evaluation (IBT) | Supplementary, Supplementary | Increased in primary liver tumors in males |
| 2-year mouse oncogenicity evaluation (IBT) | Valid, Core-Minimum | Not oncogenic at 30, 1000 or 3000 ppm |
| Six month dog feeding study | Core-Minimum | NOEL = 100 ppm |
| 90-day rat feeding study | Supplementary Data | |

| | | |
|---|---|---|
| 90-day dog feeding study | Core-Minimum | NOEL = 500 ppm |
| Teratology, rat | Core-Minimum | Not fetotoxic or teratogenic at the high dose, 360 mg/kg |
| 3-generation reproduction study, rats (IBT) | Supplementary Data | No effects suggested up to 1000 ppm |
| Mouse dominant lethal study | | Negative |
| Ames Mutagenicity Assay | | Negative |
| (Summary primarily derived from Registration Standard. Standard for results of acute testing). | | See Registration |

Discussion:

Published Tolerances are as follows:

| | |
|---|---|
| Corn, grain | 0.1 ppm |
| Soybeans | 0.1 ppm |
| Meat, inc. poultry | 0.02 ppm |
| Milk and Dairy Products | 0.02 ppm |
| Eggs | 0.02 ppm |

Tolerances Reviewed by Toxicology Branch but not yet published are as follows:

| | |
|---|---|
| Sorghum grain | 0.3 ppm |
| Sorghum forage and fodder | 2.0 ppm |
| Peanuts | 0.1 ppm |
| Peanut hulls | 1.0 ppm |
| Peanut forage and hay | 3.0 ppm |

Tolerance held in abeyance by request of registrant:

| | |
|---|---|
| Potatoes | 0.1 ppm |

The corn, soybean, meat, milk and egg tolerances were established conditionally based upon the following requirements:

1. Re-evaluation of histopathology from the 90-day dog study would be submitted by 3/15/79.

2. The two-year rat study was to be submitted by 3/15/79.

3. No additional tolerances would be considered until the aforementioned studies were reviewed and accepted.

4. Ciba-Geigy will repeat their IBT mouse oncogenic study, although it is classified as Valid.

5. Ciba-Geigy agreed to initiate a 6 month dog study.

The aforementioned studies have all either been initiated or submitted. A repeat of the rat chronic/oncogenicity study is also underway. The only other additional data lacking, but desirable, is a 3-generation reproduction study to replace the IBT study classified as Supplementary Data. Per the Registration Standard for Metolachlor (September, 1980), a teratology study in a second species, was conducted and submitted (See Review, below).

The Allowable Daily Intake (ADI) is calculated as .0013 mg/kg/day in the Registration Standard, based on a NOEL of 100 ppm (2.5 mg/kg/day) in a six month dog study and a 2000 fold safety factor. It is noted that the safety factor which is currently used by Toxicology Branch for tolerances based on six month dog studies is 1000 fold rather than 2000 fold. Thus it is recommended, that the ADI for Metolachlor be changed to .0026 mg/kg/day based on a 1000 fold rather than a 2000 fold safety factor.

The Theroetical Maximum Residue Contribution (TMRC) from existing tolerances is 0.01712 mg/day/1.5 kg diet, including sorghum and peanuts, per the Registration Standard. The requested tolerances will contribute .019185 mg/day/1.5 kg diet additional residue.

A new TMRC, taking into account existing and requested tolerances, would thus be .036305 mg/day/1.5 kg of diet. This utilizes approximately 23.27% of the revised ADI.

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.

2. Six Month Interim Report of a Two-Year Oral Feeding Study of Metolachlor in Rats, conducted at Raltech Scientific Services and submitted by Ciba-Geigy on January 28, 1981.

   Albino CD rats are being fed either 0, 30, 300 or 3000 ppm in the diet. Seventy rats per group were started on test and at least 60 rats remain in each test group. Mean body weight of high dose males was slightly less than that of controls, low or mid dose males (586 vs. 597, 614, and 624, respectively) at Week 26. Mean body weight of high dose females was significantly less than that of controls, low or mid dose females (319 vs. 343, 344 and 346). No other compound related effects were observed with two possible exceptions - SGOT appears to be decreased in a dose-related fashion in both males and females and SGPT appears to be decreased in both high dose males and females.

   It is noted that SGOT was also significantly decreased ( $p < .05$) in 3000 ppm males at the 6-month measurement in the previous Two-Year Oral Feeding Study in Rats, conducted at IBT (discussed below).

3. Two-Year Chronic/Oncogenicity Oral Toxicity Study in Albino Rats, performed at Industrial Biotest Laboratories Study No. 622-07926, Decatur, Illinois, February 9, 1979 and submitted by Ciba-Geigy Agricultural Division.

   (This study has been validated and is classified as Supplementary Data as both a chronic feeding study and as an oncogenicity study).

   Sixty male and 60 female Charles River strain albino rats were fed diet containing 0, 30, 300, 1000 and 3000 ppm of CGA-24705 Technical. Feeding was initiated when animals were 29 days of age. Animals were housed individually in wire-bottomed steel cages and diet was performed by the blending of test compound in a high speed blender. Batch#FL-750227 (99.9% purity) was used during week 1-29 and Batch#FL-752105 was used for all following weeks. Diet preparation records support weekly diet preparation with the exceptions of periods between January 2 and March 3, 1976 (8 weeks) and between May 4 and July 15, 1976 (9 weeks). Diet samples were collected at months 0, 3, 6, 12, 15, 17, 18 and 20 and weekly for the remainder of the study. Food consumption data were collected from 20 rats per sex week for 13 weeks and monthly thereafter.

Each animal was weighed weekly for 13 weeks and monthly thereafter. Observations were recorded sporadically. Blood and urine samples were taken after 3, 6, 12, 18 and 24 months of testing from 20 rats per sex of the control and high dose groups. Ten additional animals per sex were on test in the control and high dose groups to month 12, at which time the animals were removed from test and either immediately sacrificed (5 animals per sex per group) or allowed a one month recovery period prior to sacrifice. Blood was analyzed for total leukocyte count, erythrocyte count, hemoglobin concentration, hematocrit, differential leukocyte count, platelet count, MCV, MCH, MCUC, SAP, SGPT, SGOT, BUN, Glucose, total cholesterol and total protein. Urine was analyzed for glucose, albumin, pH, specific gravity, and microscopic elements.

Gross and microscopic examinations were performed on all animals unless precluded by autolysis. Tissues examined included:

Adrenals
Aorta (thoracic)
Brain (cerebrum, cerebellum, pons)
Caecum
Colon
Epididymides
Esophagus
Eyes with optic nerves
Femur
Gonads
Heart
Kidneys
Liver
Lung
Lymph nodes (cervical, mediastinal, mesenteric)
Mammary gland
Muscle (skeletal)
Pancreas
Parathyroid glands
Peripheral nerve (sciatic)
Pituitary gland
Prostate gland
Salivary gland (submaxillary) sublingual, parotid)
Small intestine (duodenum, jejunum, ileum)
Spinal cord
Spleen
Sterum (bone marrow)
Stomach (cardia, fundus, pylorus)
Thyroid glands
Trachea
Urinary bladder
Uterus

Results:

(Diet analysis records indicate that all test groups ingested diet containing somewhat less test compound than was intended. Time weighted average levels of CGA-24705 in the diet were 27.1, 254.5, 945.1 and 2457.1 ppm for the 30, 300, 1000 and 3000 ppm targeted levels (See memo of August 14, 1979 by L. Anderson). Although this per se does not effect the validity of the study or it's results, it should be taken into account in the association of given levels of test compound with toxicological effects.)

The mean body weight of high dose males was significantly ($p < .05$) less than controls from month 18 to study termination and sporadically less than controls prior to month 18. Female mean body weight of the high dose group was significantly less than controls from month 21 to study termination and sporadically less than controls prior to month 18. Group T-III female mean body weight were sporadically less than control females. Mean body weights of other test groups were similar to controls.

Food consumption of all male and female test groups were comparable to their corresponding controls.

Clinical chemistry, hematology and urinalysis of high dose males and females (the only test groups examined) were unremarkable with the exception of SGOT in males, which was significantly ($p < .05$) less than controls at the 3, 6 and 12 month measurements. This parameter was not significantly effected at the 18 and 24 month measurements.

The histopathology and organ weights of the 12 month sacrifice animals were unremarkable compared with control animals.

Among the T-IV animals removed from test at month 12 and allowed a 4 week recovery period, only the thyroid to body weight ratios were remarkable. The thyroid to body weight ratio was significantly less ($p < .01$) in females and slightly less in males. Absolute thyroid weight was not effected by treatment, suggesting that the decreased thyroid to body weight ratios are likely the result of a decreased body weight in high dose animals.

Among final sacrifice animals, the brain to body weight ratios of both males and females were significantly increased ($p < .05$). Absolute spleens weight were significantly less than controls in T-II, T-III and T-IV males. T-III female spleen was significantly less than control and T-IV female spleen weight was slightly less than controls. Liver weight comparisons were unremarkable.

No treatment related gross lesions were evident. Histopathologic lesions suggestive of treatment-related effects were found only in the liver. Focal hepatocellular hypertrophy and hyperplasia incidences were similar in all groups. Curiously, extramedullary hematopoiesis incidences appeared to be decreased among treated animals. Incidences were 16, 4, 12, 6 and 5 of 0, 30, 300, 1000 and 3000 ppm males and 20, 15, 14, 18 and 5 of the 0, 30, 300, 1000 and 3000 ppm males were diagnosed as having focal extramedullary hematopoiesis of the liver.

One or more "Hypertrophic-hyperplastic nodules" were found in the liver of 2/55, 2/58, 0/55, 4/51 and 5/54 males of 0, 30, 300, 1000 and 3000 ppm dose groups, respectively.

Among females, the incidence was 1/54, 1/59, 3/60, 3/60 and 9/60 in the 0, 30, 300, 1000 and 3000 ppm groups.

Among other neoplastic lesions, only two types were remarkable and both of these were found in the liver of females. Cystic cholangioma was found in the liver of 2/54, 2/58, 1/60, 2/60 and 6/60 of the 0, 30, 300, 1000 and 3000 ppm groups respectively. Hepatocellular carcinoma, a relatively rare tumor in females of this strain of rat, was found in two high dose females and not in any other group of females. Histopathologic diagnoses of male and female liver lesions are summarized in the following tables:

Table 1
Primary Liver Tumors in Females

| | PPM | | | | |
|---|---|---|---|---|---|
| | 0 | 30 | 300 | 1000 | 3000 |
| Hypertrophic-Hyperplastic nodule | 0 | 1 | 2 | 1 | 3 |
| Hypertrophic-Hyperplastic nodules | 1 | 0 | 1 | 2 | 6 |
| Angiosarcoma | 0 | 0 | 0 | 0 | 1 |
| Cholangioma | 0 | 0 | 1 | 0 | 0 |
| Cystic cholangioma | 2 | 2 | 1 | 2 | 6 |
| Hepatocellular carcinoma | 0 | 0 | 0 | 0 | 2 |
| Total (# animals with primary liver tumors) | 3 | 3 | 5 | 5 | 15* |
| Number examined (month 13-Final sacrifice) | 54 | 58 | 60 | 60 | 60 |

*Three animals each bore two primary liver tumors.

Table 2
Primary Liver Lesions in Males

| | PPM | | | | |
|---|---|---|---|---|---|
| | 0 | 30 | 300 | 1000 | 3000 |
| Hypertrophic-Hyperplastic nodule | 1 | 0 | 2 | 2 | 1 |
| Hypertrophic-Hyperplastic nodules | 0 | 0 | 0 | 0 | 1 |
| Angiosarcoma | 0 | 0 | 1 | 0 | 0 |
| Hepatocellular carcinoma | 2 | 1 | 1 | 1 | 3 |
| Total (# animals with primary liver tumors) | 3 | 1 | 4 | 3 | 5 |
| Number examined (month 13-Final sacrifice) | 55 | 55 | 54 | 50 | 54 |

Thus an increase in the incidence of primary liver tumors is found only in high dose females. The probability that this increase is due to chance is small (P <.005) and the variety of forms of tumor expression in the liver suggest that though the liver is a target organ, a variety of cell types and locations may be effected within the liver.

Core-Classification: Supplementary Data. A NOEL was not established. Validation deficiencies are presented in the memos of December 17, 1979 and August 14, 1979 from L. Anderson.