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

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

WL Bu 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.

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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).

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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-

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)

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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.

Reviewed by: D. Stephen Saunders, Ph.D.

Section Head: Quang Bui, Ph.D.

Data Evaluation Record

Study Type: Mutagenicity- *In vitro* gene mutation assay

Study Title: L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenicity Test.

EPA ID nos.: A.I. code 100-587
Rec. No. 173279
Accession #262713
Caswell #188DD
TOX PN #1878 (1986)

Sponsor: Ciba Geigy Corp.
Agricultural Division
Greensburg, N.C.

Testing Laboratory: Ciba Geigy ltd.
GU 2.3 Experimental Pathology

Study number: 831500

Study Date: 12-5-84

Study Authors: Beilstein, P. and Muller, D.

Test Material: Metolachlor (CGA 705) technical, batch op. 303010, 95.9% a.i.

Dosages: 15.6-1000 nl/ml range-finding
9.5-190 nl/ml without activation
10.5-210 nl/ml with activation (1st test)
56-280 nl/ml with activation (2nd test)

Positive control: Ethylmethane sulfonate (without activation)
Dimethylnitrosamine (with activation).

Vehicle control: 1% DMSO in culture medium.

Test system: Cultured L5178Y/TK$^{+/-}$ mouse lymphoma cells, harvested in exponential growth phase.

Study objective: "Evaluation of any property of the substance or its derivatives to induce point mutations."

Methods

Mouse lymphoma TK+/- cells obtained from growing cultures were cleansed of spontaneous TK-/- mutants by incubation with a mixture of thymidine, hypoxanthine, methotrexate and glycine (THMG) for 24 hours. Cells were then incubated an additional 3 days with a mixture of thymidine, hypoxanthine and glycine before use in the experiment.

About 3 x 10^5 cells/ml were incubated with the various concentrations of test chemicals for 4 hours, in the presence or absence of metabolic activation. The incubation temperature was not specified. Test concentrations were selected on the basis of cell viability in a preliminary range-finding assay. After treatment with the test material, cells were washed, and incubated for 3 days to allow expression of forward TK-/- mutations. Cells were counted daily and density was adjusted to maintain 3 x 10^5 cells/ml. At the end of the expression period, 4 x 10^5 cells were inoculated into 5 ml of semi-solid agar containing 50 ug/ml of BUdR (5-bromodeoxyuridine) and incubated for 14 days for determination of mutants (8 tubes/test concentration). Cell viability was assessed by inoculation of 200 cells/5 ml agar, and incubation for 11 days in the absence of BUdR (4 tubes/test concentration). At the end of the incubation periods, the number of colonies in each tube was counted, and counts were normalized to 100% viability. Results were expressed as the number of TK-/- mutants/ 10^6 cells.

Results

The preliminary range-finding assay demonstrated that concentrations of 250 nl/ml and higher completely inhibited cell growth. On the basis of these results, concentrations of 9.5 to 190 nl/ml were tested in the absence of metabolic activation, and concentrations of 10.5 to 280 nl/ml were studied in the presence of metabolic activation (two studies, see below).

No effect on the incidence of mutations was noted after incubation of metolachlor in the presence or absence of metabolic activation (Tables 2-4, photocopied from the study report). The positive controls induced the expected mutagenic response, demonstrating the sensitivity of the test system.

Conclusions

Under the conditions of the test, metolachlor does not induce a mutagenic response.

Classification: Acceptable

Metolachlor toxicology review

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___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

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Reviewed by: D. Stephen Saunders, Ph.D.

Section Head: Quang Bui, Ph.D.

Data Evaluation Record

Study Type: Metabolism- rat

Study Title: New feces metabolites of CGA 24 705 in the rat.

EPA ID nos.: A.I. code 100-587
Rec. No. 173279
Accession #262713
Caswell #188DD
TOX PN #1878 (1986)

Sponsor: Ciba Geigy Corp.
Agricultural Division
Greensburg, N.C.

Testing Laboratory: Ciba Geigy ltd.
Agricultural Division
Basle, Switzerland

Study number: 26/81

Study Date: 8-20-81

Study Authors: Mucke, W.

Test Material: Metolachlor (CGA 705) technical, universal ring 14-C label, 43.0 uCi/mg S.A., >99% purity

Dosages: 29.9 mg/kg in ethanol/PEG 200/water mixture by gavage.

Test animal: Female Tif:RAI[SPF] rats, 20/group.

Methods

Twenty female rats were administered a single administration of 5.74 mg of 14-C-metolachlor. The average body weight of the test group was 192 grams, consequently the average dosage was 29.9 mg/kg. Urine and feces were collected over the next 3 days. Feces from day 1 and 2 were pooled and metolachlor metabolites were extracted by a variety of techniques. Metabolites were then identified by TLC, NMR and mass spectrometry.

Results

A total of 89.9% of the administered radioactivity was excreted over 3 days, and elimination of label was about equal

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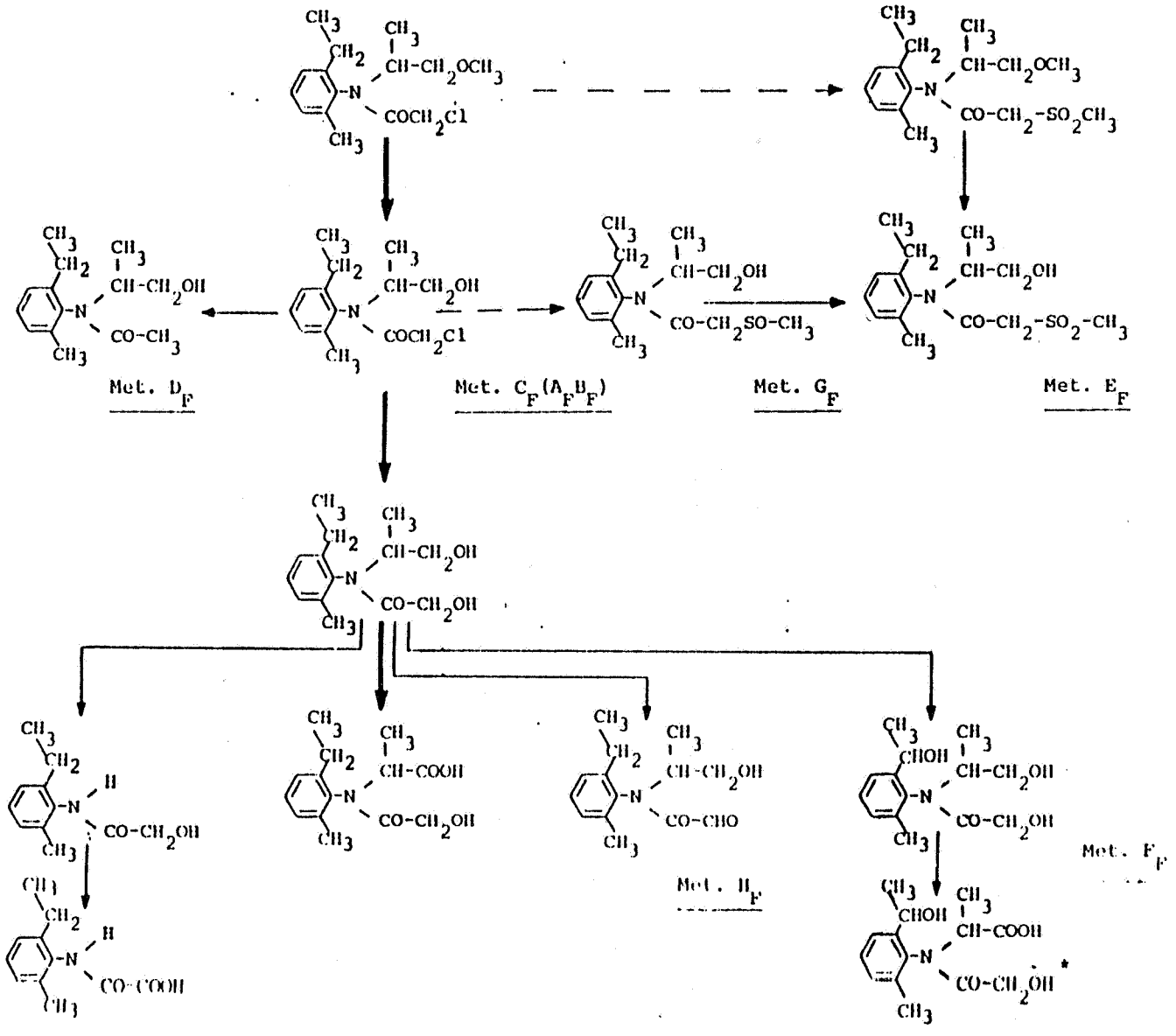
between urine and feces. The pooled feces samples from days 1 and 2 accounted for about 46% of the administered radioactivity. In general, the identified metabolites accounted for only a small percentage of the administered radioactivity. The major portion of radioactivity (12%) was contained in fraction 1, and was unidentified other than as being the most polar metabolite(s). The study report noted that with inclusion of the data from the present study, 46% of the administered dose had been identified as various metabolites. The proposed metabolic pathway of metolachlor is provided in Figure 1 (photocopied from the submitted study report).

Classification: Core-Minimum (However this study does not satisfy guideline requirements unless considered with other data previously submitted.

Figure 3 Proposed metabolic pathways of CGA 24 705 in the rat



*) This metabolite was isolated previously (4) as an artifact, i.e. the lactone of the side chain dehydrated compound, believed to be formed during isolation from the [illegible]