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

18 MAR 1979

OFFICE OF
RESEARCH AND DEVELOPMENT

SUBJECT: Diallate RPAR Rebuttal Response

FROM: for Roy E. Albert, M.D., Chairman R. E. McGaughy
Carcinogen Assessment Group

TO: Bipin Gandhi, Project Manager
Office of Special Pesticide Review

THRU: Jerry Moore, CAG Liaison
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In response to your request, the Carcinogen Assessment Group has reviewed the rebuttal comments submitted by Monsanto Company to the RPAR of Diallate. In the final analysis, there is sufficient evidence to indicate a carcinogenic response associated with exposure to Diallate.

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(GAP)

The Carcinogen Assessment Group (CAG) has reviewed the rebuttal comments submitted by Monsanto Company to the RPAR of Diallate. Each rebuttal issue is summarized below followed by the CAG's response.

I. National Cancer Institute (NCI) Rat Study (Litton Bionetics)

Rebuttal Comment

(1) Monsanto Company noted errors in the data cited in the CAG report and, therefore, have presented a re-tabulation of the raw data in the rebuttal.

CAG Response

The CAG has re-evaluated the raw data and has corrected the CAG report accordingly. The CAG's revised tabulation of the data exhibits some differences from the data presented by Monsanto. It is apparent that most of these differences are due to the classification of gliomas and leukemias as sarcomas by the CAG and as carcinomas by Monsanto.

Rebuttal Comment

(2) According to their analysis of the data, Monsanto Company states that there is no statistical difference in the number of malignant tumors in the treated male rats (both dose groups combined) compared to pooled controls (13/52 treated vs. 11/64 controls, $p = .21$). There is also no statistical difference in the number of malignant tumors or of sarcomas in either treated group of male rats (high or

low dose) compared to control groups. It is acknowledged that there is an increased incidence of carcinomas in the high dose male group compared to the controls, but the incidence in the low dose male group was not different from either control group.

CAG Response

The CAG analyzes tumor incidence from each treatment group individually, whereas Monsanto Company combined the data from the high and low dose groups. Following the CAG's re-evaluation of the data, it is observed that there is a statistically significant increase in total malignant tumors in male rats of the high dose group compared to pooled controls (10/26 treated vs. 11/64 controls; $p = .032$) and also compared to one of the matched control groups (10/26 treated vs. 4/32 controls; $p = .023$).

Rebuttal Comment

(3) From their analysis of the data for female rats, Monsanto Company concludes that there is no statistically significant increase in carcinomas, sarcomas, or total malignant tumors in either treated group.

CAG Response

According to the CAG's revised tabulation of the data, it is observed that there is a statistically significant increase of carcinomas in the female rats of the high-dose group compared to pooled controls (5/26 treated vs. 3/64 controls; $p = .042$) There is no significant difference between the number of total tumors (carcinomas and sarcomas) in either treated group compared to the number of tumors in either of the matched control groups or pooled controls.

Rebuttal Comment

(4) Monsanto Company concludes that there is no statistical difference in the number of tumor bearing animals in the Diallate-treated male or female groups compared to each of the appropriate matched control groups.

CAG Response

There is a statistically significant increase of total malignant tumors in the female rats of the high dose group compared to one of the matched control groups (10/26 treated vs. 4/32 control; $p = .023$).

Rebuttal Comment

(5) Monsanto Company states that the number of tumor-bearing rats in each Diallate-treated group is lower than in either control group, and emphasizes that the high dose group has the lowest number of animals with tumors.

CAG Response

On the contrary, even according to Table 1, Appendix B1 in Monsanto Company's Rebuttal, the data indicate that the number of male rats with malignant tumors in the high dose group exceeds the incidence in the control groups. The incidence tabulated by the CAG was 4/32 (13%) and 7/32(22%) in the two control groups, compared with 10/26 (38%) and 4/26 (15%) in the high and low dose groups, respectively.

Rebuttal Comment

(6) Monsanto Company states that the evaluation of individual tumor types/sites is necessary to conclude that a compound is carcinogenic, and that there was no apparent effect of Diallate on the formation of individual tumor types.

CAG Response

Although there was no statisticlly significant incidence of individual tumor types, there was a statistically significant increase of total malignant tumors in male rats of the high dose group relative to pooled controls and to one of the matched control groups, and a statistically significant increase of carcinomas in female rats of the high dose group compared to pooled controls. The use of total tumor data in evaluating carcinogenicity is discussed in a recent IRLG document as follows.

"At the present time there is considerable uncertainty about the interpretation of carcinogenic responses in terms of the total tumor yield in contrast to the response in terms of a statistically significant increase of tumors in specific target organs or tissues. Traditionally, carcinogens have been recognized in human and animal studies by a decisive increase in tumors of target organs. However, it is conceivable that a generalized increase in total tumor yield, in the absence of an excess incidence in one or more target tissues, could occur, for example by a promoting effect that generally increases the spontaneous incidence of tumors in test animals or by the action of a multipotent carcinogen whose response did not reach statistical significance in any one organ even at the maximum tolerated dose."[1]

1) Interagency Regulatory Liaison Group (IRLG), Scientific Bases for Identifying Potential Carcinogens and Estimating Their Risks. February 6, 1979.

II. Industrial Bio-Test (IBT) Rat Study (Sponsored by Monsanto Co.)

Rebuttal Comment

(1) Monsanto Company observed errors in the data cited in the CAG report and, therefore, have presented a re-tabulation of the raw data in the rebuttal. It is concluded that there are no statistically significant differences in the number of animals with tumors in the Diallate-treated groups compared to the controls.

CAG Response

The CAG has re-evaluated the raw data and has corrected the CAG report accordingly. The revised tabulation agrees with that presented by Monsanto Company in the rebuttal. The data indicate that there is no statistically significant increase of total tumors (benign and malignant) or of tumors of any anatomical site in any Diallate-treated group of male rats compared to controls. Female rats treated with 100 ppm of Diallate (middle dose) showed a statistically significant increase of benign mammary tumors ($p = .021$) and, hence, of total mammary gland tumors.

Rebuttal Comment

(2) Monsanto Company states that there was no statistically significant increase in the number of mammary tumors in the treated female rats of the high or low dose group compared to the controls. Although the middle dose group showed a statistically significant increase of mammary tumors, Monsanto Company concludes that this is a random event

since there was no dose response. Furthermore, there is no statistically significant increase of mammary carcinomas.

CAG Response

As noted in the rebuttal, the female rats of the middle dose group exhibited a statistically significant increase of total mammary tumors ($p = .021$), which was attributable to the statistically significant increase of benign mammary tumors ($p = .021$) since there was no significant increase of malignant mammary tumors. This increase of benign mammary tumors in the middle dose group may indicate an adverse effect, although the possibility that this response may be a random event cannot be unequivocally refuted.

Rebuttal Comment

Monsanto Company states that there is a high spontaneous incidence of pituitary adenomas in this study. There is no apparent significant increase in the incidence of this tumor in any treated group, nor any linear increase by dose observed.

CAG Response

The CAG agrees with this conclusion.

III. NCI Mouse Study (Innes Study)

Rebuttal Comment

(1) Monsanto Company claims that the Innes study is invalid for the following reasons.

a) Only one dose level (MTD) was used in the study. The rebuttal states that the MTD has been re-defined allowing lower doses for the MTD.

b) The experimental design necessitated the dosing of litter-mates. The rebuttal states that biological and statistical significance cannot be drawn from this poor experimental design. It is suggested that a bias of inherited tendencies (e.g., predisposition to hepatoma formation) cannot be eliminated because the litter mates were not randomly distributed among the test groups.

CAG Response

a) The MTD (maximum tolerated dose) is defined in the NCI Guidelines as "..the highest dose of the test agent given during the chronic study that can be predicted not to alter the animal's normal longevity from effects other than carcinogenicity."[2] Innes et al.[3] reported that the MTD was selected on the basis of a series of studies where the

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2) NCI Guidelines for Carcinogen Bioassay in Small Rodents, NCI Tech. Report Ser. No. 1, Feb. 1976, p. 14 U.S. Dept. of Health, Education and Welfare, PHS, NIH, NCI-CG-TR-1.

3) Innes, J.R.M. et al. (1969) Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note. J. Nat. Cancer Inst. Vol. 42, p. 1102.

maximal level given in a single dose, in 6 daily and in 19 daily doses resulted in zero mortality. A comparison of the survival data in the carcinogenicity study indicates that the number of 18-month survivors in the Avadex (Diallate)-treated groups was similar to that of the vehicle (0.5% gelatin) control and untreated control groups for each respective species/sex. Hence, the doses given in the study were tolerated by the treated animals and, therefore, did not exceed the MTD.

b) In this study, treated mice were administerd Avadex (Diallate) (in 0.5% gelatin) by stomach tube from 7 days to 4 weeks of age. Thereafter, the mice were weaned and were given Diallate (without a vehicle) in the diet. Since the study began prior to weaning, each test group (e.g., Diallate-treated, positive or negative control group) was comprised of litter-mates, instead of a random assortment of litter-mates which was precluded by the study design.

During the MRAK Commission review of this study which recommended that human exposure to Diallate be minimized,[4] Mr. Carrol Weil presented a dissenting opinion which included criticism of the non-random allocation of animals (which is cited in Monsanto's rebuttal). The report of

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4) Report of the Secretary's Commission of Pesticides and Their Relationship to Environmental Health, Parts I and II. U.S. Dept. of Health, Education and Welfare, Washington, D.C. (1969) p. 470.

the MRAK Commission states that each of the issues presented by Mr. Weil were discussed and resolved. Specifically, in response to Mr. Weil's criticism of non-randomization, the MRAK Commission reported that "...the data were reexamined on a litter basis, in keeping with the Epstein-Mantel approach, rather than on the single-animal-basis employed in the Journal of the National Cancer Institute report. All compounds which had been judged positive for tumor induction (significant at the 0.01 level, or stronger) remained positive."[5] Thus, although non-randomization of litter-mates is not the optimal experimental design, there is no evidence in this study that a bias existed for a genetic predispositon to tumor occurrence.

Rebuttal Comment

(2) Monsanto Company acknowledges the statistically significant increase in hepatomas in male mice in this study, but considers the apparent sex-specificity unusual.

CAG Response

The data from the Innes study indicate a statistically significant increase in hepatomas in both strains of male mice when compared with either their respective matched (vehicle) control, negative control, or pooled negative

5) Ibid p. 483

control groups; and a statistically significant incidence in female mice of strain X when compared with the pooled negative control group only. Contrary to the statement by Monsanto Company, it is known that hepatic tumors occur more frequently in male mice than in females.[6]

Rebuttal Comment

(3) Monsanto Company states that there was no statistically significant increase in pulmonary adenomas in either sex of either strain of mouse, whereas the CAG report indicated a small statistically significant increase of lung adenomas in both sexes of strain X and in males of strain Y.

CAG Response

Upon re-analysis of the data, there is a borderline statistically significant increase of pulmonary adenomas in the Diallate-treated males of strain X compared with the matched (vehicle) control group ($p = .051$) and with the pooled negative control groups ($p = .041$).

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6) Stewart, H.L. (1976) Comparative aspects of certain cancers. Chpt. 10 in: Cancer, A Comprehensive Treatise, Vol. 4, F.F. Becker (ed.), Plenum Press, New York.

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