DB-123
TXR-4289

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

004289

Nov 7 1978

DATE. November 6, 1978

SUBJECT: Goal 2E, PP#8G2028, 9H5199. Request for establishment of temporary tolerances of 0.05 ppm in or on cottonseed and 0.2 ppm in refined cottonseed oil for residues of the herbicide oxyfluorfen and its metabolites containing the diphenyl ether linkage. Caswell ~~#138AAA~~ EPA Registration#707-EUP-97

FROM: William Dykstra, Ph.D
Toxicology Branch/HED TS-769 WHD 11/7/78

TO: Libby Welch, Team 25 & Residue Chemistry Branch
Special Registrations

Chemistry Branch Considerations:



Recommendations:

1. In the 20 month mouse feeding study with RH-2915 (goal technical), the high-dose males showed a statistically significant increase in hepatic hyperplastic nodules (report of Dr. Newberne). Hyperplastic nodules are considered as evidence of tumor formation (benign). This consideration may trigger an oncogenic RPAR criterion. The registrant is requested to inquire from Dr. Newberne and other pathologists why hyperplastic nodules are not considered as neoplasms or tumor formation in their opinions.

2. The various technicals evaluated toxicologically are considered similar enough to be representative of the presently manufactured goal technical. No additional toxicological evaluation of the currently maunfactured goal technical is required.

3. Toxicological evaluation of intermediates and by-products of goal technical shows that all of the compounds tested are similar in toxicity to the active ingredient of technicals previously tested.

4. Toxicology Branch defers to Residue Chemistry Branch regarding the results and adequacy of analytical procedures for detection of azoxy-goal or azo-goal.

5. Toxicology Branch defers to CAG regarding the results and acceptability of Carcinogenic risks to applicators and consumers from exposure to perchloroethylene in goal technical and goal 2E.

6. The memo of 1/24/78 from W. Dykstra to Libby Zink is no longer applicable and will be revised pending resolution of recommendations #1, 4 and 5.

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EPA FORM 1320-6 (REV. 3-76)

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Review

Twenty month dietary feeding study in mice (International Research and Development Corporation, Final Report 4/25/77; submitted by Rohm and Haas, Co. 11/15/77.

Test Material: The compound was a yellow brown chunky powder and was identified as RH-2915, Technical AI (85.7%), Lot. PL7518006.

Three hundred male (weighing from 22 to 33 grams) and 300 female (weighing from 19 to 28 grams) Charles River CD-1 mice were used in this study. The mice were housed individually in hanging wire mesh cages and were maintained in a temperature, humdity and light controlled room during the study. Water and the control and test diets were available ad libitum. Following 12 months of compound administration, 5 male and 5 female mice from each control group and 200 ppm dosage level were sacrificed and necropsied. All the surviving male mice (19) at the 2 ppm dosage level were sacrificed and necropsied at 18 months of study. All reamining mice were sacrificed and necropsied at 20 months of study. Tissues were collected for histopathology.

RH-2915 Technical was fed in the diet at dosage levels of 2, 20 and 200 ppm. During study weeks 57 and 58 the mice at the 200 ppm dosage level were fed 800 ppm. Sixty male and 60 female mice were used at each dosage level and also in 2 control groups.

The dosage levels were calculated on an active ingredient basis, so that a factor of 1.17 was used in all calculations. The compound was mixed with a small amount of basal diet (ground purina laboratory chow) in a mortar with pestle. This premix was then mixed with the total amount of diet in a twin shell blender. The first control group received the basal diet only on the same regimen as treated mice. The second control group received the basal diet with absolute ethanol added at a concentration of 1.36 ml/kg for the first 56 weeks of study and at a concentration of 4.08 ml/kg thereafter. The ethanol was premixed with the basal diet in a mortar with a pestle and then with the total amount of diet in a twin shell blender. The mice were observed daily for changes in general behavior and appearance. Detialed observations were recorded weekly. Individual body weights were recorded weekly for the first 11 weeks of study and monthly thereafter. Sex group food consumption was recorded weekly. At termination blood samples were obtained from 6 mice/sex/group for analysis.

Hematological studies included hematocrit, hemoglobin, total erythrocyte count, total and differential leucocyte counts and coagulation time.

Biochemical studies inculded fasting glucose, urea nitrogen, serum total protein, albumin, serum glutamic pyruvic transaminase activity, serum alkaline phosphatase activity and prothrombin time. Albumin to globulin ratios were calculated. Necropsy and tissue examination were performed on all mice.



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Results

No changes in general behavior and appearence considered to be related to compound were observed. A nodule was noted for a few mice at each dosage level including the control groups. Survival (No surviving/No initiated less interim sacrifice and missing mice) at 20 months of study was as follows:

| | No. surviving/no. initiated | |
|---|---|---|
| | Male | Female |
| Control 1 | 20/55 | 27/55 |
| Control 2 (ethanol) | 23/55 | 23/55 |
| 2 ppm | 19/59* | 25/60 |
| 20 ppm | 22/60 | 25/60 |
| 200 ppm | 28/55 | 31/54 |

*Sacrificed at 77 weeks.

Changes in body weight were similar for control and treated mice. Food consumption was similar for control and treated mice.

No changes considered to be related to the compound were seen in the hematological studies.

No changes considered to be related to the compound were seen in the biochemical values was considered to be related to the age of the mice.

Increased liver weights relative to body weight (48%) were seen for male mice that received 200 ppm of RH-2915 for 20 months. No treatment related gross lesions were seen in any of the mice from the experimental group which died or were sacrificed during the second year of the study. There was no statistically significant increase in the incidence of any tumor in any RH-2915 feeding group when compared to either control group. Statistically significant increases in the incidence of the following histopathologic changes were seen: hepatocellular regeneration (males receiving 200 ppm), liver necrosis (males receiving 20 and 200 ppm, females receiving 20 ppm), portal lymphocytic infiltration (male receiving 200 ppm) and amyloidosis of the lung and myocardium (male receiving 200 ppm). These findings indicate that RH-2915 had an adverse effect the mouse liver at diet concentrations of 20 and 200 ppm. The significance of the amyloidosis, which is commonly seen in older mice, is not apparent.

The unusual hepatocellular regeneration described as involving the entire liver to about the same degree and "characterized microscopically as an overall marke variation in the size of hepatocytes with an overall rather high rate of mitotic activity", was judged not to be neoplastic. However, the pathologist stated that "the possibility of its being a preneoplastic lesion warrants consideration".

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Since there was a question concerning the nature of the regenrative changes, a second pathologist, Dr. Paul Newberne of the Department of Nutrition and Food Science, Massachusetts Institute of Technology was asked to make a histologic evaluation of the livers of the mice that died or were sacrificed during the seond year of the study. Dr. Newberne's report is being submitted along with the IRDC report.

Dr. Newberne reported the presence of "nuclear and cellular enlargement, nuclear abnormalities and mild necrosis of hepatocytes in male mice fed 20 ppm and in male and female mice fed 200 ppm" RH-2915. He found no statistically significant increase in tumor incidence in any group but did find a statistically significant increase in the incidence of "nodules of atypical hepatocytes" in male mice fed 200 ppm and sacrificed at the end of the study.

Both pathologist are in agreement that the administration of RH-2915 did not produce a statistically significant increase in the incidence of hepatocarcinoma. The same conclusion is obtained when the data from the two pathologists is combined. IRDC found a higher incidence of hepatocellular regeneration in male mice that received 200 ppm of RH-2915 and Dr. Newberne found a higher incidence of hyperplastic nodules in this same group. The difference in the findings was not entirely the result of different diagnosis being given to the same mouse but was due, to a larger degree, to a difference in the criteria used by the two pathologists.

Conclusions:

The discrepancies in histopathological interpretation of liver cell effects by IRDC and Dr. Newberne of MIT need to be resolved. IRDC interpreted the liver effects in the high-dose males (200 ppm) as non-neoplastic regenerative changes. Dr. Newberne interpreted these same liver effects as hyperplastic nodules. Hyperplastic nodules are often referred to in the open literature as neoplastic nodules, indicating tumor formation. Other effects observed in the high-dose males were increased relative liver weight. The incidence of other neoplasms (excluding the liver) was not increased by RH-2915 administration. No adverse effects were produced in mice that received the 2 ppm diet concentration. The NOEL for systemic toxicity is 2 ppm in the diet contingent upon resolution of the liver effects interpretation.

Classification: Core-Minimum Data

TOX/HED:th:REngler:11-6-78

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RH-2915 Herbicide: Twenty Month Mouse Dietary Feeding Study - Incidence of (5)

Regeneration, Hyperplastic Nodules, and Hepatocellular Carcinoma in Mice Dying

Between Weeks 53-87 and in those Sacrificed Terminally (87 Weeks) - Based on Diagnosis by IRDC

| Group | Dosage ppm | Regeneration 53-87 | Regeneration 87 | Regeneration Total | Hyperplastic Nodule 53-57 | Hyperplastic Nodule 87 | Hyperplastic Nodule Total | Hepatocellular Carcinoma 53-57 | Hepatocellular Carcinoma 87 | Hepatocellular Carcinoma Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MALES | | | | | |
| IA | 0 (untreated) | 0/17 | 0/21 | 0/38 | 1/17[a] | 1/21 | 2/38 | 0/17 | 2/21 | 2/38 |
| IB | 0 (ethanol) | 0/17 | 0/22 | 0/39 | 0/17 | 0/22 | 0/39 | 1/17 | 1/22 | 2/39 |
| II[b] | 2 | 0/25 | 0/18 | 0/43 | 1/25 | 0/18 | 1/43 | 0/25 | 0/18 | 0/43 |
| III | 20 | 1/21 | 0/21 | 1/42 | 0/21 | 2/21 | 2/42 | 1/21 | 2/21 | 3/42 |
| IV | 200 | 3/17 | 11/27 | 14/44[c,d,e] | 1/17 | 0/27 | 1/44 | 0/17 | 7/27 | 7/44 |
| | | | | | Females | | | | | |
| IA | 0 (untreated) | 0/13 | 0/28 | 0/41 | 0/13 | 0/28 | 0/41 | 0/13 | 1/28 | 1/41 |
| IB | 0 (ethanol) | 0/16 | 0/24 | 0/40 | 0/16 | 0/24 | 0/40 | 0/16 | 0/24 | 0/40 |
| II | 2 | 0/21 | 0/25 | 0/46 | 0/21 | 0/25 | 0/46 | 0/21 | 0/25 | 0/46 |
| III | 20 | 0/22 | 0/24 | 0/46 | 0/22 | 1/24 | 1/46 | 0/22 | 0/24 | 0/46 |
| IV | 200 | 0/13 | 3/31 | 3/44 | 0/13 | 4/31 | 4/44[e] | 0/13 | 0/31 | 0/44 |

[a] One animal diagnosed as having an adenoma.

[b] Group II males sacrificed at week 77.

[c] Statistically significant difference ($P < 0.05$) from Control Group I-A.

[d] " " " " " " " I-B.

[e] " " " " " Control Groups I-A amd I-B pooled. Chi Square with Yates correction factor.

RH-2915 Herbicide: Twenty Month Mouse Dietary Feeding Study - Incidence of Regeneration, Hyperplastic Nodules, and Hepatocellular Carcinoma in Mice Dying Between Weeks 53-87 and in those Sacrificed Terminally (87 Weeks) - Based on Diagnosis by MIT

| Group | Dosage ppm | Regeneration 53-87 | Regeneration 87 | Regeneration Total | Hyperplastic Nodule 53-57 | Hyperplastic Nodule 87 | Hyperplastic Nodule Total | Hepatocellular Carcinoma 53-87 | Hepatocellular Carcinoma 87 | Hepatocellular Carcinoma Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MALES | | | | | |
| IA | 0 (untreated) | 0/17 | 0/21 | 0/38 | 1/17 | 2/21 | 3/38 | 0/17 | 0/21 | 0/38 |
| IB | 0 (ethanol) | 0/17 | 0/22 | 0/39 | 0/17 | 1/22 | 1/39 | 1/17 | 0/22 | 0/39 |
| II | 2 | 0/25 | 0/18 | 0/43 | 2/25 | 0/18 | 2/43 | 0/25 | 0/18 | 0/43 |
| III | 20 | 0/22 | 0/21 | 0/43 | 0/22 | 4/21 | 4/43 | 1/22 | 0/21 | 1/43 |
| IV | 200 | 0/14 | 0/27 | 0/41 | 1/14 | 10/27[b,c] | 11/41[b,c] | 0/14 | 3/27 | 3/41 |
| | | | | | FEMALES | | | | | |
| IA | 0 (untreated) | 0/13 | 0/28 | 0/41 | 0/13 | 1/28 | 1/41 | 0/13 | 0/28 | 0/41 |
| IB | 0 (ethanol) | 0/14 | 0/24 | 0/38 | 0/14 | 1/24 | 1/38 | 0/14 | 0/24 | 0/38 |
| II | 2 | 0/20 | 0/25 | 0/45 | 0/20 | 2/25 | 2/45 | 0/20 | 0/25 | 0/45 |
| III | 20 | 0/22 | 0/24 | 0/46 | 0/22 | 1/24 | 1/46 | 0/22 | 0/24 | 0/46 |
| IV | 200 | 0/13 | 0/31 | 0/44 | 0/13 | 4/31 | 4/44 | 0/13 | 0/31 | 0/44 |

[a] Group II males sacrificed at week 77.

[b] Statistically significant difference ($P < 0.05$) from Control Group I-B

[c] " " " " " Control Groups I-A and I-B pooled. Chi Square with Yates correction factor.