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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: EPA Reg. No. 3125-58; Disulfoton: Subacute Inhalation Study in the Rat
Caswell No. 341

TO: George LaRocca
Product Manager (15)
Registration Division (TS-767)

THRU: Christine F. Chaisson, Ph.D. C.F. Chaisson 11/6/84
Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: George Z. Ghali, Ph.D. G. Ghali 10/25/84
Toxicology Branch
Hazard Evaluation Division (TS-769)

Registrant: Mobay Chemical Corporation
Kansas City, MO

Action Requested:

Evaluation of a subacute inhalation toxicity study in the rat: An IBT replacement.

Conclusions and Recommendations:

The treatment caused a decrease in body weights and mortality of 15 and 50% in females of the 3.1 and 3.7 mg/m^3 groups, respectively. Plasma cholinesterase was inhibited at all concentrations. A NOEL could not be established. The study is classified as core-supplementary .

DATA EVALUATION RECORD

STUDY TYPE: Subacute inhalation study in rats.

CITATION: Thyssen, J. and Mohr, U. Disulfoton (S 276), the active ingredient of ^{R}DI-SYSTON, subacute inhalation study on rats. An unpublished report (Bayer No. 9065, Mobay ACD No. 69361) prepared by Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, West Germany, for Mobay Chemical Corporation. April 1, 1980.

ACCESSION NUMBER: 072293.

LABORATORY: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Federal Republic of Germany, and Department of Experimental Pathology, Medizinische Hochschule, Hannover.

QUALITY ASSURANCE STATEMENT: Not present for this report.

TEST MATERIAL: Disulfoton (S 276, the active ingredient of DI-SYSTON^{R}), batch no. 808609112, Lot No. 1968, purity was 94.4 percent.

PROTOCOL: Toxicity experiments described below encompass two studies (Study I and II). Both studies were performed using Wistar TNO/W74 rats; the objectives of Study II was to determine a NOEL, which could not be determined in Study I, and to reproduce the results observed in female rats of study I.

Study I (No. S276/002):

1. Three groups of albino rats, each consisted of 10 animals/sex, with body weight range of 170-220 g at the beginning of the study, were exposed for 6 hours/day, 5 days/week, for three weeks to a nominal concentration of 0.5, 2.5, and 12.5 mg disulfoton/m^3. The test compound was "formulated in an ethanol/Lutrol mixture (1:1)." A fourth group of 10 animals/sex was similarly exposed to the solvent only at a concentration of 20 ml/m^3.

2. The test compound was "dynamically nebulized" and animals were exposed in such a way that "no skin contact with the aerosols" occurred. The concentration of disulfoton in the exposure chamber was determined by gas chromatography "10 duplicate determinations." Actual concentrations were 0.1, 0.5, and 3.7 mg/m^3, corresponding to the nominal concentrations of 0.5, 2.5 and 12.5 mg/m^3, respectively. Particle size distribution was determined only once using cascade impactor; 73.9 to 90.8 percent of the particles were 1.5 µ or less, and 96.0 to 99.7 percent were 3.0 µ or less in size.

3. During the study animals were housed in Type III Makrolon cages (5 rats/cage) and were kept at approximately 21° C and 12 hours light/dark cycles.

4. Animals were observed daily for mortality and behavioral changes. Body weights were determined before exposure and weekly thereafter.

5. Twenty-four hours after the final exposure, 5 animals/sex/group were anesthetized by ether and blood was obtained by cardiac puncture for hematology and clinical chemistry tests. Hematology parameters studies included hematocrit, hemoglobin, mean corpuscular volume, erythrocyte and thrombocyte counts, and total and differential leukocytes counts. Clinical chemistry parameters included SGOT, SGPT, alkaline phosphatase, plasma urea, and blood glucose levels.

6. Urine was collected from 5 animals/sex/group for 16 hours during the third week of exposure, and was analyzed for glucose, hemaglobin, albumin, urobilinogen and pH, and was examined microscopically.

7. Erythrocyte - and plasma-cholinesterase activity was determined before exposure and weekly thereafter on blood samples obtained from the retroorbital plexus using 5 animals/sex/group. Brain cholinesterase activity was determined at the end of the study using 5 animals/sex/group.

8. Twenty-four hours after the last exposure, animals were anesthetized by ether, exsanguinated, and gross pathology was performed. Absolute and relative organ weights were determined for the following organs: thyroid, heart, lungs, liver, spleen, kidneys, adrenals and testes/ovaries.

9. Histopathologic examination was performed on tissues from 5 animals/sex/group. These tissues included: heart, lungs, liver, kidneys, spleen, thyroid, adrenal, testes, esophagus, stomach, ovaries, eyes, bronchial lymph nodes, trachea, larynx, head, and bone marrow.

10. Statistical analysis was performed using the non-parametric Wilcoxon method to compare the means.

Study II (No. S276/003):

1. In this study a group of 10 rats/sex, and another group of 20 female rats were exposed to disulfoton at nominal concentration of 0.1 and 12.5 mg/m^3, respectively, for 6 hours/day, 5 days/week for 3 weeks. Analytical concentrations of the test compound in the exposure chamber were 0.02 and 3.1 mg/m^3, respectively. Another control group of 10 rats/sex was similarly exposed to 20 ml/m^3 of the solvent (ethanol:Lutrol mixture 1:1).

2. Housing, feeding, and exposure method of animals, as well as particle size determination, observations, hematologic and clinical chemistry

parameters, urinalyses, gross necropsy, organ weights, and histopathologic examination were the same as in Study I. About 92 to 95 percent of the particles were 1.5 μ or less in size.

3. Plasma-, erythrocyte-, and brain-cholinesterase activities were determined in animals that were exposed to 0.02 mg/m^3 only.

RESULTS:

Study I

Observations and Mortality: Animals in the highest dose group (3.7 mg/m^3) had muscular tremors, convulsions, increased salivation and "difficult breathing" during the first week (females), and at the end of the first week (males). Rats exposed to the intermediate dose level (0.5 mg/m^3) were lethargic; this effect was manifested on the second (females), and third (males) weeks of exposure. Both male and female animals in the lowest dose group were lethargic for "a brief time" after exposure during the third week of the study.

Five female rats from the highest dose group died during the study: one after each of the third, fourth, and 12th exposure, and two following the 10th exposure.

Body Weights: Body weights of male and female rats exposed to 0.1 and 0.5 mg/m^3 were comparable to that of the control group throughout the study. Animals exposed to 3.7 mg/m^3 of the test compound had lower body weights than the control group throughout the study (Table 1).

Hematology: No significant difference in hematologic parameters studied was found between the control and exposed animals.

TABLE 1. Mean Body Weights of Rats Exposed to Sulfoton for 3 Weeks

Group	Sex	Mean Body Weights (g) Week 0	Week 1	Week 2	Week 3
Control	M	199	196	202	207
3.7 mg/m^3	M	196	192	190	192
Control	F	175	173	173	173
3.7 mg/m^3	F	171	153	154	165

Clinical Chemistry: No significant change was observed in the clinical chemistry parameters studied except alkaline phosphatase which was increased in all exposed female animals as compared to the control. Females exposed to 0.1, 0.5, and 3.7 mg/m^3 of Disulfoton showed an

increase in alkaline phosphatase averaging 9.1, 16.8, and 36.0 percent of the control value, respectively. However, examination of the individual animal data showed that clinical chemistry values in the highest concentration group were reported for only one animal.

Urinalyses: Urinalyses showed no test compound-related effect.

Cholinesterase Activity: Both male and female animals exposed to 3.7 mg/m^3 of disulfoton showed a decrease in plasma-, erythrocyte-, and brain-cholinesterase activity. In addition, females exposed to 0.5 mg/m^3 showed a decrease in plasma-, and brain-cholinesterase activity. Plasma cholinesterase activity was also decreased in females exposed to 0.1 mg/m^3 (Table 2). As shown in Table 2 female animals were more sensitive to the inhibitory effect of Disulfoton on cholinesterase activity than males.

TABLE 2. Percent Decrease in Cholinesterase Activity of Exposed Animals as Compared to Pre-exposure Values*

Cholinesterase Activity in	Dose Level mg/m^3	Sex	Percent Decrease at Day 5	14	21
Plasma	3.7	M	67.3	80.0	70.9
	0.1	F	36.8	51.9	42.1
	0.5	F	47.0	51.7	44.4
	3.7	F	93.4	96.7	92.9
Erythrocyte	3.7	M	18.2	29.2	27.7
	3.7	F	28.3	32.0	39.7
Brain	3.7	M	-	-	47.5
	0.5	F	-	-	30.1
	3.7	F	-	-	57.7

* Changes in brain cholinesterase activity as compared to that of the control.

Gross Pathology: Female animals that died during the study showed mottled, distended lungs; pale discoloration of the kidneys, bloated gastrointestinal tract; and ulcer-like foci in gastric mucosa. Animals sacrificed at the end of the study did not show test compound-related gross changes.

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Organ Weights: No statistically significant compound-related effect was observed in the absolute or relative organ weights. An increase in the absolute adrenal gland weights averaging 14 percent of the control value was reported in the females exposed to 3.7 mg/m^3 of the test compound. This increase was ascribed by the authors to the test compound. However, because the increase was not dose-related and the mean adrenal gland weights at the highest dose level were calculated using data from 5 animals only (versus 10 at other dose levels), it is the opinion of this reviewer that this effect was not test compound-related.

Histopathology: An increase in the round cells, peribronchial round cells, and perivascular round cells infiltrates was observed in male and female animals of all groups, and particularly in those exposed to 0.5 and 3.7 mg/m^3 of the test material. Female animals exposed to 0.5 and 3.7 mg/m^3 of the test compound showed "reactive bone marrow changes in the form of eosinophilia, toxic granulation, and nuclear abnormalities as well as increased plasmacytes." It should be noted that tissues from only one female animal of the high dose group were examined.

Study II

Observations and Mortality: Female rats exposed to 3.1 mg/m^3 of the test compound showed signs of "inhibition of CHE activity" similar to those observed in Study I. One animal from the same group died after the 8th exposure and 2 more died after the 15th exposure.

Body Weights: Mean body weights of female rats exposed to the 3.1 mg/m^3 concentration were less than their initial body weights but not significantly different from the control.

Hematology: Except for a decrease in lymphocytes, averaging 22 percent of the control value, and an increase in polymorphnuclear leukocytes, averaging 225 percent of the control value in females at the 3.1 mg/m^3 level, no other significant change in hema- tology parameters was observed.

Clinical Chemistry: An increase in alkaline phosphatase level in female rats exposed to the 3.1 mg/m^3 level averaging 19.2 percent of the control value was observed. This increase, however, was not statistically significant. No changes were observed in other clinical chemistry parameters studied.

Urinalysis: No test compound-related effect was observed.

Cholinesterase Activity: The decrease in plasma-, and erythrocyte-cholinesterase activity in females exposed to 0.02 mg/m^3 was evident especially at the 5-day test period (Table 3). The decrease in plasma cholinesterase activity observed at day 5 in exposed females averaged 53.5 percent, control females showed a similar decrease averaging 26.8 percent of the pre-exposure value.

Gross Pathology: Female animals that died during the study showed distended lungs with dark discoloration. One animal showed erythematous

gastrointestinal tract. Sacrificed rats did not show any change that could be attributed to the test compound.

TABLE 3. Percent Changes in Cholinesterase Activity in Animals Exposed to the Test Compound at 0.02 mg/m^3 Level as Compared to Pre-exposure Values*

Cholinesterase Activity in	Sex	Percent Change at Day		
		~~7~~	~~14~~	~~21~~
Plasma	M	-27.6	-20.7	-6.9
	F	-53.5	-14.9	+7.9
Erythrocyte	M	-8.2	-4.8	-8.5
	F	-26.5	-16.8	-19.7
Brain	M	-	-	+10.9
	F	-	-	+9.6

* Changes in brain cholinesterse activity as compared to that of the control.

Organ Weights: Increases in the absolute weights of lungs, liver, kidney and adrenals of females exposed to the 3.1 mg/m^3 level was observed. These increases averaged 9.1, 13.0, 6.6, and 9.8 percent of the control values, respectively. However, these increases were not statistically significant, and could be attributed to biological variations.

Histopathology: Inflammatory changes in the lungs and reactive bone marrow changes similar to those observed in Study I were noticed in females exposed to the 3.1 mg/m^3 level.

DISCUSSION:

In this report, experiments performed were divided into two studies: Study I in which groups of Wistar rats were exposed to 0.1, 0.5 or 3.7 mg/m^3 of Disulfoton, for 6 hours/day, 5 days/week, for three weeks. Because a NOEL could not be established, the study was repeated (Study II) with animals exposed to 0.02 mg/m^3, and 3.1 mg/m^3 of Disulfoton.

In both studies, animals exposed to the highest concentration showed a decrease in body weights as compared to the pre-exposure weights and control groups. The decrease in body weights was test-compound related. No data on food consumption were given.

Female rats were more susceptible to the effects of Disulfoton than males. This was based on the following: 1) the decrease in body weights due to the test compound was more pronounced in females, 2) 50 percent and 15 percent of females exposed to 3.7 and 3.1 mg/m^3, respectively, died during the study, 3) cholinesterase inhibition was more pronounced and occurred at lower dose levels in females than males, 4) reactive bone marrow changes were more severe in female animals.

The observed increase in alkaline phosphatase in females at all dose levels could be attributed to the changes in bone marrow since no histopathologic changes in the liver were observed. These effects on alkaline phosphatase and bone marrow were reproduced in Study II at the 3.1 mg/m^3 concentration. Furthermore, changes in differential leukocyte count reported in the second study were considered by the authors as a "first signs of response...to the inflammation phenomena in the respiratory tract...and to the bone marrow changes."

In the first study, plasma-cholinesterase activity was decreased at all dose levels (0.1, 0.5, 3.7 mg/m^3) especially in female animals. Therefore, one group of animals in the second study was exposed to 0.02 mg/m^3 in order to establish a NOEL. A decrease in plasma-cholinesterase activity averaging 27.6 percent and 53.5 percent was observed on day 5 of exposure in male and female animals, respectively. Similar decrease in erythrocyte-cholinesterase activity was also observed. Although these effects showed some reversal by the 15th day, 0.02 mg/m^3 cannot be considered a NOEL as concluded by the authors.

CONCLUSIONS:

Exposure of Wistar TNO/W74 rats to Disulfoton in concentrations of 0.02, 0.1, 0.5, 3.1, or 3.7 mg/m^3 for 6 hours/day, 5 days/week for three weeks resulted in a decrease in body weights at 3.1 and 3.7 mg/m^3, and mortality rates of 15 percent and 50 percent in females, respectively. Plasma cholinesterase activity was inhibited at all concentrations; erythrocyte- and brain-cholinesterase activity was also inhibited at the higher concentrations, especially in female animals. Alkaline phosphatase levels were increased in female animals in all groups except for the group that was exposed to the lowest (0.02 mg/m^3) concentration. This effect could be attributed to the "reactive bone marrow changes" that were observed on histological examination of females; no liver lesions were observed. Inflammatory changes were also observed on histological examination of the lungs. The increase in polymorphnuclear leukocytes and the decrease in leukocytes in female animals were attributed to the changes in the lungs and bone marrow.

Since all concentrations of Disulfoton used, including the lowest concentration (0.02 mg/m^3), resulted in an inhibition of plasma cholinesterase activity, a NOEL could not be established from this study.

CORE CLASSIFICATION: Supplementary.