E. Long

May 20, 1971

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MEMO

TO: Mr. Drew Baker

Request for Additional Toxicological Data for Dithianon, Pesticide Petition No. 1F1055.

Attached is Dr. Eleanor Long's review of the pathological findings submitted by petitioner for the fungicide Dithianon. In the summary and recommendations for rats she has requested that the petitioner do some additional microscopic examination of the lesions described.

Would you please advise the petitioner that we need this additional data before we can finalize our toxicological evaluation of the safety of Dithianon. Petitioner is requesting a tolerance of 7 ppm on apples.

Clara H. Williams

Clara Williams, Ph.D.
Pesticide Tolerance Division
Toxicology Branch

Attachment

cc: OGFitzhugh
JCCummings
PRD/EPA
Atlanta Branch (Lewis)
Perrine Branch
Division Reading File
Branch Reading File
PP No. 1F1055

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TO: Mr. Drew Baker, Petitions Control Branch

FROM: Dr. Eleanor L. Long

SUBJECT: Dithianon, Review of Pathological Findings in Chronic Toxicity Studies

Pesticide Petition No. 1F1055
Thompson-Hayward Chemical Company
Kansas City, Kansas 66110
(AF 19-302)

Note: Other toxicological data have been reviewed by Dr. Clara Williams.

I. Two Year Feeding Study in Rats

TABLE I

Group	PPM Fed	Rats Begun M	Rats Begun F	Rats Surviving 88 Wks. M	Rats Surviving 88 Wks. F	Rats Surviving 104 Wks. M	Rats Surviving 104 Wks. F	Microscopic Exam. 26 Wks.	Microscopic Exam. 52 Wks.	Microscopic Exam. 104 Wks. M	Microscopic Exam. 104 Wks. F
1	0	35	35	16	14	9	9	10	10	25	25
2	20	35	35	14	20	8	12	0	0	17	13
3	200	35	35	12	14	9	10	0	0	16	15
4	1000	35	35	7	15	0	0	10	10	25	25

A. Methods

This study was done by Huntingdon Research Centre, Huntingdon, England. A total of 280 young Charles River CD rats, 35 males and 35 females per group, received either 0, 20, 200, or 1000 ppm Dithianon in the diet. All were treated for 2 years except: (1) those which died or were killed in a moribund state in the interim, (2) 5 males and 5 females per group sacrificed after 26 and 52 weeks, and (3) the remaining surviving rats on 1000 ppm which were killed because of poor clinical condition, the males at 88 weeks and the females at 96 weeks. A complete gross autopsy was performed on all animals, and all were stated to have been examined microscopically except those in 2 and 3 sacrificed at 26 and 52 weeks, and terminally at 104 weeks. Table I shows the number studied microscopically in each group at each time interval. Organs thus microscopically routinely

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examined in survivors and those sacrificed at 26 and 52 weeks were adrenals, brain, colon, duodenum, eyes, heart, ileum, kidneys, liver, lungs, cervical and mesenteric lymph nodes, ovaries, pancreas, pituitary, salivary glands, seminal vesicles, skeletal muscles, spleen, stomach, testes, thymus, thyroid, bladder, and uterus. Organs less often subjected to microscopy were aorta, cecum, femoral marrow smears, esophagus, prostate, sciatic nerve, skin, and tongue.

B. Effects

The only definite effect was a slight increase in chronic nephritis (termed "glomerulonephrosis" in this study), a spontaneous disease found in varying degree in almost all old rats, after 2 years in the high-dose females, with the average amount being 0.4 or very slight in the controls versus 1.0 or slight at 1000 ppm. Approximately the same amount (also around grade 1) was seen in all 4 male groups, but the disease is nearly always more severe in males. However, the presence of twice as much protein in the urine of both sexes at the high dose as in the controls suggests that there may also have been an increase in the males as well as in the females. Unfortunately, I cannot be absolutely certain of the amount in either sex as my figures are based upon the assumption that kidneys of all animals which died were studied microscopially, and it is not entirely clear from the data submitted that this was the case. If this was a genuine effect, considered in conjunction with the depression of growth, decreased food consumption, and tendency toward anemia which were noted by Dr. Williams, it would help explain the increased mortality in both sexes at the 1000 ppm level. There did not appear to be an effect on the liver; while a few treated livers contained considerable amounts of fat, this was also true of a similar number of controls.

While the approximately equal incidence of animals with tumor in the 1000 ppm and control groups (respectively, 7 and 11 for the males and 16 and 17 for the females) would appear to rule out the possibility of a tumorigenic effect, a closer look shows that there is a possibility that the compound may be not only a tumorigen but a carcinogen. The data, however, are inconclusive for the following reasons. (1) A major fault in this study was failure to house the rats singly. As a result of housing 5 to a cage, there was cannibalization in all groups. Needless to say, it is impossible to know how many of these cannibalized rats had tumors or other lesions. (2) The true tumor incidence at the lower and middle levels (groups 2 and 3) is unknown, as

only a small number of those thus grossly diagnosed were examined microscopically. While the gross description of most of these lesions is strongly suggestive of benign tumors (though a few were undoubtedly malignant), to definitely eliminate the possibility of malignancy, microscopy is essential. (3) Malignant tumor was diagnosed microscopically in 6 female rats at the high dose but in none of the female controls. This alone would label the chemical as a carcinogen were it not for the fact that 2 lesions at 0 ppm and one at 1000 ppm suspected grossly of being tumor were not examined microscopically, and, as already mentioned, the true tumor incidence at 20 and 200 ppm is not known. It is important to know the true incidence at each level, as this is the only way to determine the presence or absence of a linear dose response. (4) Except for a malignant lymphoma (a tumor notorious for appearing early in life), which was found in a ten-week control, the earliest malignant tumor was a fibrosarcoma in a 1000 ppm female at 6 months. (5) Even if factors 1, 2, and 3 were absent, the possibility exists that the high spontaneous incidence of tumor in the controls, especially in the females, in which 19 out of 35 were thus affected, might have masked a tumorigenic effect of Dithianon. The Mrak Commission recommended testing for carcinogenicity in at least 2 species. It is clear that the use of a second rodent species is indicated in such a case as this, in which the results in the first species were equivocal. Although dogs were also treated for 2 years with this compound, this cannot be regarded as a true carcinogenicity experiment in this species, as for this at least 6-7 years (approximately half the life span of the dog) and many more dogs than were used here are required.

C. Recommendations

1. In order to answer the question discussed above of a possible carcinogenic effect of Dithianon, it is first necessary to have microscopic diagnoses of all lesions grossly suspected of being neoplastic. Thus, I should like to request microscopic diagnoses on such lesions on which gross descriptions only have been thus far supplied from the following rats. (a) 20 ppm males - 36, 43, 45, 52 (liver and spleen); (b) 200 ppm males - 79, 81, 83, 93, 100; (c) 1000 ppm males - 112; (d) control females - 152 (pituitary), 158 (pancreas); (e) 20 ppm females - 177, 178, 181, 186, 187, 188, 191, 192, 193, 195, 198, 199 (pituitary, liver, mammary gland); (f) 200 ppm females - 228 (nodule in lung),

212, 216, 217, 222, 224 (uterine nodule), 225 (spleen), 229, 230, 231, 233; (g) 1000 ppm females - 252 (uterus), 262 (thoracic tumor), 273 (uterus); and (h) any other gross lesion in this category I may have inadvertently omitted.

2. As a corollary I should like answers to these questions. (a) Was the hepatoma in 1000 ppm male 114 benign or malignant? (b) In control female 150 was the tumor of the pars intermedia of the pituitary benign? (c) What was the nature of the pituitary tumor called simply "tumor" in control female 151?

3. In order to determine whether chronic nephritis was increased in the treated rats, it will also be necessary for me to ask for the individual rat numbers of the animals whose kidneys were examined microscopically.

4. Study of the additional data just requested may show that Dithianon is a definite carcinogen, but I do not think the present experiment is adequate to completely eliminate this possibility. If the data should still prove equivocal, I suggest that, as the Mrak Commission recommended, a second and better 2-year toxicity study be done, either on another strain of rat (preferably one without such a high spontaneous tumor incidence) or another species of rodent, such as mouse, hamster, or gerbil. Animals should be housed singly, and as a minimum, microscopy be performed on all gross lesions, all livers and kidneys, and all other organs in which there is a clinical, biochemical or hematologic indication of an effect.

II. Two-Year Feeding Study in dogs (Huntingdon Research Centre.)

A. Methods and Effects

Young beagles, 4 males and 4 females per group, were treated with 0, 40, 400, or 1000 ppm of Dithianon, which was mixed into the diet. Pathological changes attributable to the pesticide were limited to the liver. The average amount of damage was moderate at 1000 ppm, with all 8 dogs showing significant changes. These consisted of: (1) moderately severe variation in hepatic cell size in 3, (2) moderate to marked hepatic cell enlargement (midzonal, centrilobular, or focal) in 4, (3) moderate to marked deposition of brown pigment within macrophages, generally periportal, and with associated leukocytic infiltration of similar degree in 6, (4) slight to marked fibrosis with bile duct

proliferation in 2, (5) moderately severe architectural irregularity in 3, (6) irregular nodularity in 3, and (7) early cirrhosis with nodular hyperplasia in one. The liver of one female on 400 ppm, which showed slight hepatic cell enlargement and focal leukocytic infiltration with macrophagic pigmentation may be regarded as mildly affected. These changes could not be well correlated with changes in SGPT and serum alkaline phosphatase. (SAP). While the 1000 ppm group as a whole showed mild elevation of SGPT, marked rises in SGPT were found only in 2 dogs (one of which also had a significantly elevated SAP), though not in others whose livers showed the same degree of pathology. The identity of the pigment remains in doubt. Schmorl's stain for lipofuscin showed a little in all animals, but this does not account for the striking increase at the high dose, and, furthermore, lipofuscin (ceroid) is characteristically pale yellow rather than brown. The absence of icterus and the normal values for serum and urinary bilirubin and urobilinogen rule out bile. The most likely possibility is hemosiderin, indicating hemolysis. Though the reticulocyte count was not increased and bone marrow smears were said to appear normal, a slight tendency toward mild normocytic, normochronic anemia at the high dose was noted not only in the dogs but also in the rats. It is possible that hemolysis was too mild and chronic to have induced an obvious increase in erythropoiesis. A simple stain for hepatic iron, which, oddly enough, was apparently not done, would have answered this question. This should be done, as any hemolytic propensity should be known in case Dithianon should eventually be approved.

B. Summary and Recommendations

Dithianon when fed to dogs for 2 years produced deleterious effects on the liver, which were moderately severe at 1000 ppm and mild at 400 ppm, with the no-effect level being 40 ppm. It is possible that the hepatic pathology was the result of very mild but chronic hemolysis. To help answer this question, I suggest, (1) stains for iron on all the dog livers, and (2) myeloid/erythroid ratios on individual bone marrow smears on all dogs. It would also be helpful to know the cellularity of sections of bone marrow but apparently none were made.

Eleanor L. Long
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