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Dithianon. Second Review of Pathological Findings in Chronic Toxicity Studies

Mr. [illegible] Taylor, Chief
Petitions Control Branch
Pesticides Tolerances Division

Pesticide Petition 1G1035

Thompson-Hayward Chemical Company
Kansas City, Kansas 66110
(4F 19-302)

In my first review (of May 20, 1971) of the pathological data on Dithianon, I asked certain questions concerning the 2-year dog and rat feeding studies. These have now been answered.

A. Two-Year Dog Feeding Study

When administered in the diet at levels of 0, 40, 400, and 1000 ppm to groups of 8 beagles each for 2 years, Dithianon induced the following clinical and laboratory abnormalities at the highest dose: (1) poor clinical conditions in 2 females, (2) slight decrease in appetite, (3) increased serum alkaline phosphatase, (4) decreased glucose-6-phosphatase in 5/8, (5) mild normocytic, normochromic anemia, and (6) loss of weight in 2/8 (but also in one control). Pathological changes attributable to the compound were moderately severe at 1000 ppm (with all 8 dogs being affected), and mild at 400 ppm (with only one mildly affected), and were limited to the liver. These consisted of: (1) moderately severe variation in hepatic cell size in 3, (2) hepatic cell enlargement, moderate to marked in 4 and slight in one (on 400 ppm), (3) deposition of brown pigment within macrophages, generally periportal and with associated leukocytic infiltration of similar degree, moderate to marked in 6 and slight in 1 (on 400 ppm), (4) slight to marked fibrosis with bile duct proliferation in 7, (5) moderately severe architectural irregularity in 3, (6) irregular nodularity in 3, and (7) early cirrhosis with nodular hyperplasia in one. As I felt that this pathology might have resulted from chronic hemolysis because of the pigment, which apparently was not bile but was otherwise unidentified, I asked for (1) stains for iron on the livers, (2) myeloid: erythroid ratios on individual bone marrow smears, and (3) the cellularity of sections from bone marrow. These procedures were carried out. The marrows were reported to show normal cellularity and myeloid: erythroid ratios, indicating the absence of hemolysis. Perl's stain for iron produced equivocal results, with 2 moderately pigmented livers (dogs 2 and 6) being moderately positive, while 3 others equally pigmented (dogs 3, 4,

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and 6) and a third only slightly so revealed only minimal amounts of iron with Perl's stain. Although the hepatic pigmentary problem has not been solved (some might even be copper or melanin), I do not feel that more study of the dog livers is absolutely necessary. In view of all the above data, it is reasonable to consider the no effect level for the dog as 40 ppm.

3. Two-Year Rat Feeding Study

Clinical and hematologic changes induced by Dithianon in Charles River CD rats fed at levels of 0, 20, 200, and 1000 ppm for 2 years were (1) increased mortality, decreased food consumption, polydipsia, and mild anemia at 1000 ppm, (2) depression of growth in both sexes at 1000 ppm and in the females at 200 ppm, and (3) transient yellow discoloration of the fur at 1000 and 200 ppm. The original data presented also suggested the possibility of 2 serious pathologic effects at the high dose: carcinogenesis and an increase in chronic nephritis. However, before either could be proved or disproved, I was of the opinion that additional microscopy was necessary and that certain data needed clarifying, and therefore asked for this information to be supplied. My questions have now been answered, and the additional microslides of gross lesions suspected of being neoplastic which I asked for have now been made and described. It is thus possible to draw certain conclusions from the total mass of data which has now been submitted.

(1) There was a definite increase in chronic nephritis (here termed "glomerulonephrosis"), a spontaneous lesion seen in varying degree in nearly all old rats and characterized by gross enlargement and granularity, and microscopic glomerular basement membrane thickening and hyalinization, tubular dilatation with hyaline casts, tubular atrophy and regeneration, and interstitial fibrosis with some lymphocytic and plasmocytic infiltration in the 1000 ppm females. Quantitatively, there was twice as much at 1000 ppm as at 0 ppm, and moderately severe or severe disease was found in 12 high-dose females but in only 7 female controls. Moreover, this can be correlated with the increase in proteinuria in this sex at this level (twice as much at 1000 ppm as at 0 ppm, or 1000 mg% versus 500 mg%, respectively, after 83 weeks of treatment), and also with the increase in mortality (after 104 weeks there were 9 surviving female controls whereas the few remaining females on the high dose had to be sacrificed at 96 weeks due to their poor condition). While a similar trend was also noted in the males in regard to mortality (the remaining males on the high dose had to be sacrificed after 84 weeks) and urinary protein, which was twice as high in the controls (2000 mg% vs. 1000, respectively), there was so much nephritis in the male controls that it was impossible to prove an increase in any treated group.

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(2) Data on neoplasms are given in Tables I through V. There was an apparent increase in malignant tumors in the high dose females (7 versus 0 controls). The fact that 5 of the 7 were fibrosarcomas, one of which arose after only 6 months of treatment, and the sixth a nephroblastoma makes me suspicious of a mild sarcomagenic effect in this sex, though there was no evidence of a similar process in the males. The incidence of other tumors was either similar in all groups, or in the case of pituitary tumors and benign mammary tumors in females, actually decreased in the high and intermediate dosage groups in comparison with the controls. Two factors which increase the difficulty in determining whether the slight increase in malignant mesodermal tumors in the high dose females is either statistically significant or indicative of the true tumor incidence are (1) the interim sacrifices of a total of 20 rats per group at 6 and 12 months, i.e., before the age at which tumors generally begin to appear (except for occasional isolated ones), and (2) the unfortunate practice of multiple housing, which led to extensive cannibalization. My personal feeling is that while this study does not prove the compound to be carcinogenic (or, more accurately, sarcomagenic, as the tumors in question are sarcomas rather than carcinomas), it has not been proved safe for this species.

For assistance in determining whether Dithianon is a sarcomagen or not I consulted Dr. John M. Weisburger, Head of the Carcinogen Screening Section, Experimental Pathology Branch, National Cancer Institute. According to the letter of April 17 which I received from him, he and his committee (also composed of Dr. L.A. Poirier and Dr. E.K. Weisburger) after reviewing the tumor data do not believe there is sufficient evidence to call this compound a carcinogen, but they agree with me in considering the study somewhat inadequate. I quote from Dr. Weisburger's letter as follows.

"May I say also that we do not believe the protocols were entirely appropriate to assess carcinogenicity. Even though the starting number of rats was small, the killing of 10 each at 1/2 year and one year left very few animals for the full 2 year study. In future studies if for other reasons animals are to be killed at intermediate points, there should be more animals started to take that into account. For important environmental agents we believe 50 males and 50 females available for the full 2 year run are essential."

In a telephone conversation with me on April 20, 1972, Dr. Weisburger also stated that he does not think a second 2-year rat study on Dithianon is necessary, unless significant amounts of the pesticide were to get beyond the peel into the pulp of the apple, the fruit on which it is intended to be used. If a new study is to be done, he recommends at least 3 dosage levels: maximum tolerated dose, approximately 1/2 this, and 0.

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DISCUSSION

According to information which I obtained from the petition and from a discussion with Mr. George Bousch of the Chemistry Branch, who has reviewed the chemical data, Dithianon remains on the apple peel, unless the fruit is washed according to regulations. If the peel is macerated or punctured, the pesticide enters the pulp and is quickly conjugated by enzymes within the fruit to a series of metabolites, whose fate, identity, and quantity have been imperfectly characterized. In view of the opinion of the National Cancer Institute that this pesticide is not a carcinogen and the evidence just cited that it is rapidly destroyed under the proposed conditions of use, I am willing to accept the verdict of the National Cancer Institute and will not require further animal toxicity data on the compound itself. With a no-effect level of 20 ppm for the rat and 40 ppm for the dog, the animal toxicity data already obtained are adequate to support the requested temporary tolerance, 7 ppm on apples intended only for the fresh fruit market.

Although a temporary tolerance can be supported, in my opinion the metabolic data are inadequate to justify a permanent tolerance. As discussed above, the metabolic data in plants (apples) is incomplete. Moreover, the metabolism in animals has not been investigated. This would seem to be particularly important for this compound as pathological effects were found in the liver of the dog but in the kidney of the rat, suggesting that the metabolic pathways may differ from one species to another. There is also a possibility that the metabolites produced in animals and man may not be identical to those produced in apples. I understand that the Chemistry Branch would like more data on residues and metabolites in apples before a permanent tolerance is granted. To this I would like to add a request for metabolic data in rats, dogs, cows, and (if possible) man. If one or more of the metabolites should prove to be significant, additional sub-acute or chronic toxicity studies on the offending compound may be necessary.

SUMMARY

The animal toxicity data on Dithianon are adequate to support a temporary tolerance of 7 ppm on apples intended only for the fresh fruit market. However, before a permanent tolerance is granted, I recommend that metabolic data be obtained from rats, dogs, cows, and, if possible, human beings. When this information and studies which I have been told the Chemistry Branch is requesting on residues and metabolites produced by enzymes in apples have been supplied, it is possible that additional animal toxicity data may also be required.

Eleanor L. Long, M.D.
Pathologist
Toxicology Branch/FTB

cc: JCCummings
FSD/FPA
Perrine Br.
Atlanta Br. (Clewis)

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TABLE I: MISCELLANEOUS DATA ON CHARLES RIVER CD RATS FED DITHIANON FOR 2 YEARS

Group	PPM	Rats Started M	Rats Started F	No. Sacrificed 26 Weeks	No. Sacrificed 52 Weeks	Two-Year Survivors M	Two-Year Survivors F	Week Term. Sacrifice M	Week Term. Sacrifice F	Growth Depression M	Growth Depression F	Increased Mortality M	Increased Mortality F	Increased Chr.Nephr. M	Increased Chr.Nephr. F	Rats With Malig.Tumor M	Rats With Malig.Tumor F
1	0	35	35	10	10	9	9	104	104	NO	No	No	No	No	No	4	0
2	20	35	35	10	10	8	12	104	104	No	No	No	No	No	No	3	2
3	200	35	35	10	10	9	10	104	104	No	Yes	No	No	No	No	3	2
4	1000	35	35	10	10	7*	10*	88	96	Yes	Yes	Yes	Yes	?	Yes	2	7

TABLE II: EARLY NEOPLASMS IN RATS TREATED ORALLY WITH DITHIANON

Group	PPM	Week First Tumor Noted Males	Week First Tumor Noted Females	Classification of Initial Tumors in Charles River CD Rats Given Dithianon Males	Females
1	0	10	52	Malignant lymphoma	Mammary fibroadenoma
2	20	76	24	Malignant lymphoma	Mammary adenocarcinoma
3	200	33	57	Pituitary chromophobe adenoma	Mammary fibroadenoma Pituitary chromophobe adenoma
4	1000	52	26	Pituitary chromophobe adenoma	Subcutaneous fibrosarcoma

*To 88 and 96 weeks, respectively.

TABLE III: TUMORS IN FEMALE CHARLES RIVER CD RATS FED DITHIANON

Group	PPM	Number Surviving 2 Years	Week First Tumor		Total Females With Tumor						Two-Year Survivors With Tumor					
			Tot.	Malig.	Tot.	Malig.	Fibro-sarcoma	Pit.	Mam. Ben.	Mult.	Tot.	Malig.	Fibro-sarcoma	Pit.	Mam. Ben.	Mult.
1	0	9	52	0	16	0	0	11	10	6	9	0	0	8	6	5
2	20	12	24	24	19	2	1	7	14	6	11	0	0	4	10	3
3	200	10	57	94	14	2	2	5	8	3	8	0	0	3	5	2
4	1000	10*	26	26	15	7	5	5	5	6	6	4	2	3	1	3

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TABLE IV: TUMORS IN MALE CHARLES RIVER CD RATS FED DITHIANON

Group	PPM	Number Surviving 2 Years	Week First Tumor		Total Males With Tumor					Two-Year Male Survivors With Tumor				
			Tot.	Malig.	Tot.	Malig.	Fibro-sarcoma	Pit.	Mult.	Tot.	Malig.	Fibro-sarcoma	Pit.	Multiple
1	0	9	10	10	12	4	1	3	2	7	1	0	3	1
2	20	8	76	76	9	3	0	3	0	4	1	0	1	0
3	200	9	33	59	8	3	1	4	1	2	0	0	1	0
4	1000	7*	52	84	7	2	1	1	0	3	1	0	0	0

Explanations:

1. "Fibrosarcoma" includes the single renal liposarcoma in a 200 PPM female.
2. *To 88 weeks (males) and 96 weeks(females).

Abbreviations:
Ben. - benign
Pit. - pituitary
Tot. - total
Mam. - mammary
Malig. - malignant
Chr.nephr. - chronic nephritis

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TABLE V: TYPES OF TUMORS OCCURRING IN CHARLES RIVER CD RATS TREATED WITH DITHIANON

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Tumor	NUMBER OF RATS WITH TUMOR							
	MALES				FEMALES			
	0 PPM	20 PPM	200 PPM	1000 PPM	0 PPM	20 PPM	200 PPM	1000 PPM
Hypophyseal chromophobe adenoma	3	3	4	1	11	7	5	5
Mammary adenoma or fibroadenoma	0	0	0	0	10	14	8	5
Mammary adenocarcinoma	0	1	0	0	0	1	0	0
Subcutaneous (including 1 mam.)fibrosarcoma	1	0	1	1	0	0	1	4
Subcutaneous fibroma and myxofibroma(1)	2	0	0	0	1	1	0	1
Cutaneous keratoacanthoma	0	1	0	0	0	0	0	0
Malignant lympho-reticular tumor	1	2	1	0	0	0	0	0
Thyroid adenoma	4	0	0	0	1	0	0	0
Thyroid carcinoma	0	0	0	1	0	0	0	0
Parathyroid adenoma	0	0	1	2	0	0	0	0
Pancreatic islet cell adenoma	1	0	0	0	0	0	0	0
Adrenal cortical adenoma	0	1	0	0	0	0	0	1
Testicular mesothelioma	1	0	0	0				
Testicular interstitial cell tumor	0	0	0	1				
Ovarian tubular adenoma					0	1	0	1
Uterine fibrosarcoma					0	1	0	0
Renal pelvic transitional cell carcinoma	0	0	1	0	0	0	0	0
Renal liposarcoma and fibrosarcoma	0	0	0	0	0	0	1	1
Nephroblastoma	0	0	0	0	0	0	0	1
Renal lipoma	0	0	1	0	0	0	1	0
Urinary bladder papilloma	0	0	0	0	0	0	0	1
Cerebral astrocytoma	1	0	0	0	0	0	0	1
Hepatoma, benign	0	1	0	1	0	0	0	0
Hepatic lymphangioma	0	0	0	0	0	0	1	0