UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 001097

SUBJECT: Daconil (2,4,5,6-Tetrachloroisophthalonitrile) and 4-Hydroxy Metabolite (4-Hydroxy-2,5,6-Trichloroisophthalonitrile). Review of Microslides of Kidneys of CFN(WI)SPF Wistar-Derived Rats Treated Four Months With Daconil.

DATE: JAN 26 1976

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TO: Dr. O. E. Paynter
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Pesticide Petition: 2F1230

Diamond Shamrock Chemical Company
1100 Superior Avenue
Cleveland, Ohio 44114

Related Petitions: 7F0599, 7G0516, 9F0734, 1F1024

Synonyms. Daconil: chlorothalonil, DAC-2787
4-Hydroxy Metabolite: DAC-3701

This is my ninth review of the fungicide Daconil, the previous ones being dated as follows: (1) January 31, 1969, (2) May 16, 1969, (3) August 3, 1969, (4) September 16, 1969, (5) February 15, 1973, (6) February 28, 1974, (7) July 3, 1974, and (8) December 6, 1974 (the 8th review was incorporated into an official memo of a conference held December 13, 1974 to Mr. Jesse Mayes, then Acting Chief of the Coordination Branch). The latest conference, which was summarized by the Product Manager, Mr. Eugene M. Wilson, was held October 21, 1975. In my fifth review I summarized the long and complicated history of this fungicide. The present memo is concerned with my review of microslides of the kidneys of rats treated 4 months by dietary administration of the parent compound, DAC-2787 (Bio/Tox study #24-201). This study was done at our request and is an attempt to determine whether certain changes seen at doses as low as 4 ppm in 2-year experiment 200-205 (done by Hazleton Laboratories, Inc., Falls Church, Virginia) represent a true effect of the compound or are only artefacts due to improper handling of the kidneys at the time of autopsy or in processing for microscopic examination.

Methods.

This study, #24-201, was performed by Bio/Tox Research Laboratories, Inc., 533 North Broadway, Spencerville, Ohio 45887. Sections of kidneys were examined microscopically by 2 pathologists employed by Diamond Shamrock Co.: (1) William M. Busey, D.V.M., Ph.D., Experimental Pathology Laboratories, Inc., Herndon, Virginia, and (2) Paul M. Newberne, D.V.M., Ph.D., Professor of Nutritional Pathology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, as well as by myself.

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DAC-2787 was fed in the diet (Peerless Laboratory Animal Diet) to 8 groups of young adult CFN(WI)SPF (Wistar-Derived) rats, the unmedicated control group consisting of 30 of each sex and each treatment group of 15 of each sex, for 4 months at levels of 0, 1, 2, 4, 15, 30, 60, or 120 ppm. Throughout the study observations were made on appearance, behavior, growth, food consumption, and survival. At the end of the test period, all the animals were killed and the kidneys removed and prepared for microscopic examination. Such evaluation was made by 2 independent pathologists, as already noted.

Results Reported by Diamond Shamrock Chemical Company.

Appearance, behavior, growth, food consumption, and survival (100%) were virtually identical for all groups, including the control. No gross or histopathologic changes which were observed could be attributed to Daconil.

My Evaluation of Microslides of Rat Kidneys, Bio/Tox Study #24-201

TABLE I.

Kidneys of Rats Treated by Diet with Daconil Four Months in Bio/Tox Study #24-201

			Chronic or Subacute Nephritis					
PPM	No. Micro.		Male		Female		Highest Grade	
Fed	M	F	Grade	No.	Grade	No.	Male	Female
0	30	30	0.13	14	0.15	14	0.5	0.75
1	15	14	0.20	9	0.07	4	1.0	0.25
2	15	15	0.20	9	0.05	3	1.0	0.25
4	15	15	0.15	8	0.05	3	0.5	0.25
15	15	15	0.05	3	0.03	2	0.25	0.25
30	14	15	0.09	5	0.08	5	0.25	0.25
60	15	15	0.12	6	0.02	1	0.5	0.25
120	15	15	0.12	7	0.08	5	0.25	0.25

Grading Scheme.

0 - normal, no lesion
0+ (0.25) - minimal
+ (0.5) - very small, very slight
1 - slight, small, mild
1-2 (1.5) - slight to moderate
2 - moderate
2-3 (2.5) - moderate to severe
3 - severe, marked
4 - the worst possible

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As Table I shows, kidneys of almost all the rats started on the experiment were submitted for histopathologic examination ("No. Micro." in the table means the number of rats examined microscopically). Though the degree of pathologic change in general is slight or less (on the average, only minimal), several types of lesions are present in both treated and control groups.

1) The most common lesion is nephritis, a prominent spontaneous disease in old rats but, in my experience, usually rare in animals as young as these. Both subacute and chronic stages are present. The subacute variety is characterized by focal interstitial and, to a lesser extent, intratubular, infiltration of various types of leukocytes (chiefly lymphocytes and small macrophages but often a few neutrophils and plasma cells also) within the cortex and, in a few cases, the medulla as well. In the chronic form this cellular infiltrate is accompanied by regeneration and (only in rare instances here) fibrosis of the affected convoluted tubules. Subacute nephritis is minimal in 4 male controls (animal numbers 58, 74, 84, and 285), 5 female controls (144, 168, 212, 255, and 259), 3 males (6, 115, and 116), and 2 females (242 and 269) on 1 ppm, 3 males (14, 48, and 132) and 1 female (170) on 2 ppm, 5 males (27, 72, 87, 102, and 125) and 3 females (155, 164, and 258) on 4 ppm, 2 males (31 and 45) and 1 female (184) on 15 ppm, 1 male (120) and 5 females (150, 160, 188, 192, and 197) on 30 ppm, 3 males (20, 26, and 35) and 1 female (204) on 60 ppm, and 1 male (67) and 3 females (216, 261, and 274) on 120 ppm. It is very slight in degree in 1 male control (126). Chronic nephritis is minimal in 9 male controls (animal numbers 94, 97, 98, 108, 112, 113, 117, 131, and 283), 6 female controls (154, 193, 225, 226, 275, and 280) 5 males (3, 18, 81, 119, and 124) and 2 females (182 and 229) on 1 ppm, 5 males (22, 41, 78, 99, and 111) and 2 females (165 and 248) on 2 ppm, 2 males (36 and 57) on 4 ppm, 1 male (70) and 1 female (272) on 15 ppm, 4 males (49, 75, 82, and 105) on 30 ppm, 2 males (50 and 118) on 60 ppm, and 6 males (5, 16, 19, 51, 91, and 92) and 2 females (138 and 232) on 120 ppm. The lesion is very slight in 2 female controls (210 and 270), 1 male on 4 ppm (1) and 1 male on 60 ppm (93). It is mild in 1 female control (243), 1 male on 1 ppm (30), and 1 male on 2 ppm (4). For each dose, the table shows the number with either subacute or chronic nephritis, the average grade, and the highest grade for each sex. The table also shows that the incidence of nephritis does not vary appreciably from one group to another and that in none of the 8 is the average degree more than minimal.

Other renal lesions are less common than nephritis but resemble it in being scattered through all 8 groups with no relation to treatment. Two of these are usually regarded as part of the chronic nephritis complex but will be considered separately here because of their relative rarity and minimal grade; they would probably have been more prominent in older animals with

farther advanced disease. They are 2) tubular distension by casts and 3) periglomerular fibrosis (glomerular capsular thickening). Tubular dilatation due to hyaline (proteinaceous) casts is seen in 3 male controls (39, 117, and 284), 2 female controls (243 and 275), 1 female on 1 ppm (152), 1 female on 2 ppm (191), 1 male on 15 ppm (31), 1 male on 30 ppm (49), 1 female on 60 ppm (178), and 1 female on 120 ppm (232). This is the sole lesion in 39, 152, 178, 191, and 284; in the others there is other evidence of nephritis. The casts are located in the collecting tubules in 39, 152, 191, 275, and 284, in the loop of Henle in 31 and 117, and in the convoluted tubules in 49, 178, 232, and 243. Periglomerular fibrosis is limited to 3 males: 133 on 2 ppm, in which there is no evidence of associated nephritis, and 102 and 75 (on 4 and 30 ppm, respectively), in which there is minimal associated nephritis. Fibrosis and hyalinization of the glomeruli themselves (and not merely of the capsule of Bowman), which are characteristic of advanced chronic murine nephritis, are not evident in the kidneys of any of these animals. 4) A fourth lesion is minimal focal infiltration of the lamina propria of the renal pelvis (with occasional extension into the epithelium) and around large blood vessels in the hilus of lymphocytes and sometimes small macrophages. This is often associated with true nephritis, and is no doubt part of it. Rats in which it is unaccompanied by other evidence of infection, however, are 1 male control (135), 3 female controls (227, 260, and 264), 1 male (103) on 1 ppm, 1 female (240) on 15 ppm, 3 males (10,47, and 86) on 30 ppm, 2 males (17 and 24) on 60 ppm, and 1 male (7) on 120 ppm. Other rare incidental lesions unrelated to treatment with Daconil are: 5) minimal focal calcium deposition in 2 ppm male 99, 2 ppm female 146, 4 ppm male 125, and 15 ppm female 272, 6) one or two foci of intimal hyperplasia of small arteries in 60 ppm male 93, and 7) a 2 mm in length subcapsular streak of hemorrhage with fibrosis in 60 ppm male 100. In summary, none of the lesions which are present in the kidneys of the rats in this 4-month experiment can be attributed to Daconil, as their incidence and severity are similar in treated and control groups.

The changes found in certain rats treated at dosages ranging from 4 through 60 ppm in previous 2-year study 200-205 (done by Hazleton Laboratories, Inc.), i.e., vacuolation and coagulation necrosis of the epithelium of the proximal tubules of the juxta-medullary segment of the renal cortex without an associated inflammatory reaction, are not evident in any of the rats in the present study (Bio/Tox 24-201). Because of this lack of inflammation, the coagulative change was suspected of being an artefact due to improper handling either at the time of autopsy or in the preparation of microslides rather than true necrosis (i.e., antemortem cell death). However, as it was seen in test animals only and levels above 120 ppm in the rat and 60 ppm in the dog have been definitely nephrotoxic, such an assumption would have been both dangerous and irresponsible. Therefore, another experiment, the present one under review (Bio/Tox 24-201), was requested. As none of

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the 268 rats whose kidneys have been studied microscopically (of a total of 270 started on the experiment) in this latest study by Bio/Tox shows either of these changes, it is safe to conclude that they were indeed artefacts and not lesions attributable to the administration of Daconil.

One further comment on the microsections in the present study is necessary. This is the fact that approximately half have been very poorly prepared. Fortunately, most of the pathological changes are minimal. If they were more serious, or if equivocal changes such as were seen in study 200-205 were present, these slides are so poor that they might be impossible to interpret. It is ironic that such poor slides should have been submitted after all the discussion about the necessity for careful handling of tissue to ensure the absence of just such artefacts as it now seems were present in experiment 200-205.

Summary and Conclusions

In conclusions, the questions raised by chronic rat study 200-205 on Daconil (2,4,5,6-Tetrachloroisophthalonitrile) have now been answered. As it has now been established that the rat kidney is unaffected by dietary administration of Daconil at levels of 1 through 60 ppm and that no other effects have been found in rats treated at these dosages for periods up to 2 years (120 ppm was also negative in the new 4-month Bio/Tox study evaluated in this review but this level has not been tested for longer periods), 60 ppm may be considered the no-effect level for this fungicide in the rat.

2 year rat NEL = 60 ppm

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