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SC 330

Petitions Control Branch (SC-13) May 16, 1969
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THRU : Dr. H.L. Richardson, Chief, Pathology Branch (SC-940)
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Daconil (tetrachloroisophthalonitrile). Also called DAC-2787.

PESTICIDE PETITION No. 7F0 743

Diamond Shamrock Company
Painesville, Ohio
(AF 25-202)

My initial review of the animal toxicity data on Daconil was on January 31, 1969. This report concerns the conference on April 4 between Drs. R.H. Blackmore and L.D. Shott representing Hazleton Laboratories, Inc. (which performed the animal studies in question for Diamond Shamrock Company), and Drs. H.L. Richardson, M.E. Richardson, K.J. Davis, and E.L. Long representing the Food and Drug Administration (Pathology Branch, Division of Pharmacology and Toxicology), and reports on additional feeding studies on rats and dogs and slides with microscopic sections of kidneys from rats treated orally for 1 and 2 years which were left with me following the conference.

At the conference, the Hazleton representatives told us that the formulation to be put on the market (Daconil-2787 Technical or DAC Technical) was being used in the animal tests currently in progress, whereas in the older studies a formulation of Daconil-2787 plus a blend of impurities (which I refer to as "DAC Mixture") was used.

Component	Per Cent	
	DAC Technical	DAC Mixture
Tetrachloroisophthalonitrile (DAC-2787)	97.6	93.6
[redacted]		

IMPURITY INFO IS NOT INCLUDED

I. Slides

A. Project 200-205, Rats, Oral Feeding of DAC-Technical, One-Year Interim Report

This is currently being fed at levels of 0, 4, 10, 20, 30, 40, and 60 ppm. I have previously reviewed the data and examined the slides of the animals killed after 3 months, and reported on both

in my memo of Jan. 31. The slides submitted at this time were from 14 animals (apparently from both sexes) on each level which had been killed after one year of treatment. My examination of these under the microscope confirmed the existence of the lesion Dr. Shott had demonstrated by microprojector at the conference. Vacuolation of the cytoplasm of the cells lining the pars recta of the proximal tubule, a lesion which first appeared in the 3-month animals at 20 ppm in the males but only 4 ppm in the females, first became apparent in these sections at 30 ppm; at this level it was seen in only 1/14 rats and was present in slight degree. At 40 ppm it was found in 6/14 and at 60 ppm in 8/14, ranging in degree from very slight to slight to moderate, and the higher degrees being associated with slight focal karyolysis and dissolution of cell membranes. While a few rats from nearly all dosage levels (including the controls) showed some chronic nephritis (characterized by glomerular hyalinization, tubular hyaline droplet degeneration, atrophy, dilatation, regeneration, fibrosis, and luminal cast formation, and interstitial lymphocytic infiltration), there was no evidence of correlation between the nephritis and the tubular vacuolation.

B. Projects 200-148 and 200-154, Rats, Oral Feeding of DAC Mixture, Two Years.

I reviewed the data on both in my first memo. In 200-148 DAC Mixture was fed at levels of 0, 1500, and 15000 ppm; in 200-154 it was fed at 0 and 5000 ppm. The slides submitted were from 6 rats on 0 ppm, 6 on 1500, and 7 on 15000 in Project 200-148, and from 10 on 0 and 10 on 5000 in Project 200-154. The lesions which I found in the treated groups were similar to those described by the Hazleton pathologist. They consisted of (1) hypertrophy of the lining epithelial cells of the pars recta of the proximal tubule associated with increased luminal diameter, resulting in an enlarged tubule, (2) these changes plus vacuolation and hyperplasia of the tubular cells, resulting in intraluminal stratification of cells, and (3) vacuolation of the tubular epithelium unassociated with the other changes. The second and third lesions were not limited to the pars recta, but seemed to be irregularly distributed through all portions of the convoluted tubules. These changes, existing either singly or in association with each other, were seen in 3/7 at 15000 ppm and 7/10 at 5000, and averaged slight to slight to moderate in degree. Questionable minimal changes of a similar nature were found at the lowest dose. Except for 2 in Project 200-148 which showed very slight tubular epithelial cell vacuolation, these alterations were absent in the controls. These results indicate that the 3 specific lesions present in the test rats must have resulted from DAC Mixture. Other changes noted in the test groups were a possible slight increase in pigment, and variable

amounts of chronic nephritis. As the latter was also prominent in the controls (and present in variable degree in all of them), it could not be ascribed to treatment, although there did seem to be more in the treated animals with the specific tubular lesions than in those lacking them.

II. New Projects

A. Project 200-198, Rats, Oral Feeding of DAC Technical for Four Months

1. Methods. Groups of 35 males and 35 females received either 0, 250, 500, 750, or 1500 ppm for 4 months. Periodic tests during life consisted of body weight, food consumption, hemograms (hematocrit, hemoglobin, erythrocyte count, and total and differential leukocyte count), urinalysis, and serum and urine sodium, potassium, and chloride; at the end of the experimental period serum protein-bound iodine was determined. After the 4-month period, 15 of each sex per group were killed and autopsied. Histopathologic examination was performed on all organs from 5 of each sex from the control and 250 ppm levels, and on the liver and kidney of the same number from each of the other levels.

2. Effects. (a) There was slight depression of growth in the females at 1500 and 750 ppm. (b) In comparison with those of the controls, the kidneys of the males at the high dose were enlarged; relative renal hypertrophy (increased percentage of body weight) was noted in both sexes at the high dose and in the males at 750 ppm also. (c) Pathology was limited to the kidneys of the treated animals. While those of the controls were within normal limits, dose-related alterations were found in all treated groups, varying in severity from slight at 250 ppm to moderate at 1500, and appearing more prominent in males than in females. They consisted primarily of swelling of the tubular epithelial cells, tubular dilatation, regeneration, and cast formation, with smaller amounts of tubular epithelial cell vacuolation, degeneration, and cytoplasmic droplet formation. There was also somewhat more pigment in the tubular epithelium at the 2 upper doses than in the controls.

B. Project 200-206, Dogs, Oral Feeding of DAC Technical, One Year Interim Report.

1. Methods. Young adult beagles, 8 of each sex per group, were put on diets supplemented by 0, 60, or 120 ppm. Clinical studies during life have consisted of periodic body weight, food consumption, hematology (hematocrit, hemoglobin, coagulation time, erythrocyte count, and total and differential

leukocyte count), blood chemistry (fasting glucose, urea nitrogen, and serum glutamic pyruvic transaminase), and urinalysis. After one year, 4 of each sex per group were killed and autopsied, and the liver, thyroid, and kidneys examined microscopically. The remaining dogs are to be kept on the experiment for a second year.

2. Effects. (a) Clinical changes included death of one high-dose dog due to accidental strangulation, transient loss of weight in another, and reddening of the skin and roughening of the fur (which improved somewhat after oral treatment with a fatty acid) in 2 others; a male control also lost 0.7 kg. (b) Urinary bilirubin was mildly elevated at the high dose. (c) Hepatic vacuolation was somewhat greater at 120 ppm than at 60 or 0; the vacuoles were considered to be glycogen (which is not abnormal), though there is no indication that special stains were done to identify them more accurately. (d) The kidneys of the treated males at both levels showed a dose-related change not found in the controls, i.e., vacuolation of the epithelium of the convoluted tubules in the deep portion of the cortex averaging moderate in degree at the high dose and slight at the low. There was more renal pigment at both test levels than in the controls, also.

DISCUSSION AND SUMMARY

The new data tend to confirm my impression, which I believe is shared by Hazleton Laboratories, Inc., that both the old (DAC Mixture) and the new (DAC Technical, which is to be put on the market) formulations of Dacouil produce significant renal pathology in both rats and dogs. On examination of the slides from the 2-year rats from both chronic experiments now completed (DAC Mixture, Projects 200-148 and 200-154) and from rats killed after one year on the one now in progress (DAC Technical, Project 200-205), I found lesions identical to those described by Hazleton. The 2-year rats (on the Mixture) showed striking changes characterized by hypertrophy and dilatation of the convoluted tubules with hyperplasia and cytoplasmic vacuolation of the lining epithelial cells. This lesion was not seen in any control and was different from the chronic nephritis found in both control and treated groups. It was present at both 15000 and 5000 ppm. There was evidence that it might have been present in lesser degree at 1500 ppm, also, and that it was associated with increased chronic nephritis, but because of the high incidence of the latter and the small number of kidneys submitted for microscopic study, it was impossible to be certain of either. Though the other and more striking changes were not found in rats given DAC Technical (the market formulation) for one year, they did show vacuolation of the cytoplasm of the cells lining the pars recta of the proximal tubule at doses as low as 30 ppm (this began to appear after only 3 months), and a lesion similar to that seen after 2 years in Projects 200-148 and 200-154 was described

by Hazleton in rats treated for only 4 months at somewhat higher levels ranging from 1500 ppm down to 250 ppm in Project 200-198. These findings strongly suggest that similar serious pathology may be found in the rats completing the 2-year period in the present chronic experiment on the new formulation (Project 200-205), and indicate the necessity for a complete gross and microscopic study of every animal remaining on the experiment, particularly of the kidneys, but also of the other organs as well. In the new 2-year dog experiment (Project 200-206), vacuolation of the convoluted tubular epithelium was also seen, and at both levels tested, 120 and 60 ppm. As the finding of similar renal pathology in both dogs and rats makes the probability of such lesions occurring in man even more likely than if only one of these species were affected, a thorough study in both animals is necessary to determine the exact no-effect level, assuming there is one. As the new formulation is somewhat different from the one previously tested and the chief ingredient in somewhat higher concentration, it is necessary to study the other organs in as much detail as the kidneys.

In summary, my recommendations for Project 200-205 in rats and Project 200-206 in dogs are similar to those I made in my first memo. (1) All organs in all rats and dogs surviving the 2-year experimental period and of all those dying at any period (except the ones markedly autolyzed) should be examined grossly and microscopically. When I requested a complete work-up only on certain organs in my first memorandum, I did not know that the formulation had been changed. (2) If vacuolation of the cytoplasm of the renal tubular cells proves to be as prominent in the 2-year animals on these projects as in those previously studied, special stains should be done to ascertain their nature - whether fatty, hydropic, or otherwise. (3) After seeing the slides, I was not as impressed by the amount of pigment as I had been after reading the description of them, as it did not appear to me to be more prominent in treated than in control rats, with the possible exception of the 2-year animals in Project 200-148. However, if pigment should prove prominent in the 2-year animals in Projects 200-205 and 200-206, its nature should be investigated.

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