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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460
February 9, 1984

OFFICE OF
RESEARCH AND DEVELOPMENT

SUBJECT: Review of the Metalaxyl Fungicide Mouse Oncogenicity Study

FROM: Bernard Haberman
Pathologist
Carcinogen Assessment Group (RD-689)

TO: William L. Burnam
Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Robert E. McGaughy
Acting Technical Director
Carcinogen Assessment Group (RD-689)

FEB 13 1984

As you requested in your January 17, 1984 memorandum, I have undertaken and completed a "rapid review" of the Ciba-Geigy mouse oncogenicity study of Metalaxyl. This reviewed material included:

A. Volume 1, Report 1 of the 2-year mouse oncogenicity study (submitted to EPA June 10, 1982).

B. Supplemental data on male mice liver tumors (dated December 12, 1983).

C. Evaluation of liver tissues from male mice by Dr. Jerry Hardisty of Experimental Pathology Laboratories Inc. [EPL] (submitted to EPA January 11, 1984).

D. Submission of supplemental data on mouse toxicology regarding the maximum tolerated dose [MTD] (dated January 18, 1984).

E. Evaluation of Tyzzer's disease and incidence of liver tumors in Metalaxyl oncogenicity study in mice (dated January 24, 1984), and

F. The microscopic slides of liver sections from the male mice (animal numbers 1 through 240) fed Metalaxyl for 2 years from Life Science Research, LTD., Essex, England.

Based upon the review of this material, several conclusions can be reached.

First, with regard to the latest review of the male mouse liver sections by Dr. Jerry Hardisty of EPL (submitted to EPA January 11, 1984), there does not appear to be a carcinogenic response in the liver to oral

administration of Metalaxyl. It was stated by Ciba-Geigy at the January 11, 1984 meeting that the EPL (Dr. Hardisty) pathology report replaced the microscopic diagnoses in the June 10, 1982 and December 12, 1983 reports (A and B). My quick review of the microscopic slides confirms this negative finding by EPL for oncogenicity, even though there were several disagreements on the individual diagnoses of some hepatocellular lesions, i.e., regarding the presence or absence of a liver tumor or the severity of a tumor. These disagreements do not alter the final conclusion that Metalaxyl did not cause an increase of liver tumors in male mice. It should be noted that many of the microscopic slides reviewed were of poor quality; their condition made interpretation of several liver lesions difficult.

Second, the presence of the so-called "Tyzzer's disease" in these mice caused me concern initially. The lesions of multifocal necrotizing inflammation in the liver can be the result of other infectious agents besides Bacillus piliformis. Dr. Hardisty of EPL mentioned at the January 11, 1984 meeting at EPA that there were confirming intestinal lesions for Tyzzer's disease, but also said no special stains were done to identify the organism. It is known that the main diagnostic feature of Tyzzer's disease is the demonstration of the Bacillus piliformis organism in the cytoplasm of apparently viable hepatocytes using special stains, such as methenamine silver or Giesma stain. It is impossible to determine the amount of interference with the interpretation of scientific data that B. piliformis has caused in past experiments. Several authors alluded to the fact that Tyzzer's disease has been responsible for more ruined cancer studies in mice than any other disease (American Journal of Pathology 64:717-732, 1971). Nonetheless, my review of Dr. Hardisty's pathology data of January 11, 1984 showed no correlation between the occurrence of the so-called "Tyzzer's disease" and the incidences of liver tumors in any of the animal groups. This was also stated by Ciba-Geigy in their January 24, 1984 evaluation. It is somewhat puzzling that this January 24, 1984 report mentions that the Tyzzer's animals had an increased mean lifespan compared to the non-Tyzzer's animals, when it is known that acute Tyzzer's disease usually causes an increased mortality in mice. Among the many factors that have been proposed for the occurrence of Tyzzer's disease are overcrowding and poor sanitation.

Third, the selection and appropriateness of the doses used in this mouse oncogencity study are questionable, i.e. was a maximum tolerated dose (MTD) reached? No 90-day subchronic study in the Swiss mice (ICI Adderly Park strain) was presented for selection of doses for the 2-year study. This mouse bioassay does not appear to be as sensitive as it could have been in detecting the carcinogenicity of Metalaxyl, because the animals probably could have tolerated a higher dose. During the chronic study, there was no evidence of any dose-related adverse toxic effect in either male or female mice, except for a small transient decrease in body weight in the highest dose group of male mice. If a dose range-finding study (a 90-day subchronic) had been done in this mouse strain (ICI Adderly Park strain of Swiss mice) before the chronic study, the investigators may have chosen a higher dose. However, 1250 ppm does

seem to be a relatively high dose, because it is about 1/5 as high as the LD_{50} in a different strain of mouse [random bred mice of the TiF MAG (SPF) strain] as presented in the Ciba-Geigy submission of January 18, 1984.

In summary, the 2-year oncogenicity study in mice orally adminstered Metalaxyl showed no significant difference in liver tumor incidence between treated and control mice, based on the EPL report of January 11, 1984. Questions concerning the effects of the so-called "Tyzzer's disease" on the liver tumor incidence have been resolved, but the occurrence of such a disease shows that the study may have been done under poor sanitation and husbandry practices. The question of whether the doses in the 2-year mouse study were appropriate for an MTD has not been resolved. There is no evidence that an MTD was actually reached, since no 90-day subchronic study was presented, although the LD_{50} levels presented by Ciba-Geigy (January 18, 1984) in another strain of mouse suggests that the 2-year dose appears high enough. Since the doses used in the 2-year oncogenicity study did not cause an increase in the incidence of liver tumors and higher doses may have caused an oncogenic response, it can be assumed that if Metalaxyl could be shown to be carcinogenic in mice, it would be of a rather low carcinogenic potency.

cc: Chris Chaisson (TS-769)
Henry Jacoby (TS-767)