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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Henry Jacoby, Product Manager (21)
Registration Division (TS-767C)

THRU: Jane Harris, Ph. D., Section Head J E Harris 9/3/86
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Roger Gardner, Toxicologist
Toxicology Branch
Hazard Evaluation Divisic (TS-769) Roger Gardner 8-28-86
Clifford Ts 9/5/86

SUBJECT: Review of a Range Finding Study in Mice with on Technical Grade Metalaxyl. EPA Reg. No. 100 601. Tox. Chem. 375AA. (Tox. Proj. No. 170)

Actions Requested

Review of the study cited in the appended Data Evaluation Record.

Conclusions

The results of the range finding study do not support the conclusion that 1250 ppm (highest dose tested) in a chronic feeding study with mice was a maximum tolerated dose (MTD).

I. Background

A Toxicology Branch Peer Review Committee meeting was held on June 21, 1985 to evaluate the long-term toxicity studies of Metalaxyl. The memorandum describing that meeting (see Engler, 1985) discussed an MTD issue associated with the chronic feeding study in mice as follows:

> ...While compound related effects (fatty infiltration of the liver) are noted at the highest dose it is very likely that the mice could have tolerated more compound without showing decreased weight gain and/or survival.
>
> In fact the registrant submitted information on the 33 day observation of a 90-day mouse study (see Appendix below for Data Evaluation Record on the final report).
>
> ...1250 ppm chosen for the long-term study represented a minimal rather than a maximal dose to be tolerated by the mice.

II. Discussion

Diets containing 0, 1250, 2500, or 5000 ppm metalaxyl were fed to male and female mice for up to 90 days. Subsequently, some of the test animals were given control diets for an additional 30 days in a recovery phase of the experiment. There were minimal liver effects at the 2500 and 5000 ppm levels in males (changes in glycogen content, fat vacuolization, and hypertrophy in hepatocytes which were not observed after three months of treatment). Liver weights were elevated in all treated animals after 90 days on the test diets, but no histopathological effects were reported for the liver of those animals. Absolute liver weights for test groups in the recovery phase of the study were comparable with those of the control group at termination.

Since many of the effects are reversible during treatment of the male mice, and since liver weights in treated mice were comparable to those of untreated mice after 30 days without metalaxyl, the effects observed in the range finding study should be considered physiological or pharmacological. These results support the conclusion of the Toxicology Branch Peer Review Committee that the highest level tested in the chronic mouse feeding study (1250 ppm) did not cause sufficiently significant toxicity in the 90-day subchronic study to be used in characterizing the 1250 ppm dose level as a maximum tolerated dose in the mouse oncogenicity study.

III. Reference

Engler, R. Memorandum dated December 31, 1985. Subject: Peer Review of Metalaxyl. Tox. Chem. No. 375AA. To: H. Jacoby, Registration Division.

APPENDIX

Data Evaluation Record for a Subchronic
Range Finding Feeding Study in Mice with Metalaxyl

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DATA EVALUATION RECORD

Metalaxyl (375AA)

Guideline §82-1 Subchronic Toxicity (Chronic/Oncogenicity Range Finding)

MRID: Unassigned

Barrow, D. M.: Ashby, R.; Fowler, J. S. L.; Finn, J. P. (1985) Metalaxyl Technical: Combination 30-Day to 90-Day Subchronic Dietary Toxicity Study in Albino Mice: Final Report: Addendum 1. Supplemental Clinical-, Macro- and Micro- Pathology. (Unpublished report no. 85/CIA064/180 received Jul 25, 1985 under 100-601; prepared by Life Sciences Research Limited., submitted by CIBA-GEIGY Corp., Greensboro, NC; Acc. Nos. 258811 and 258812)

REVIEW RESULTS:

VALID X INVALID ____ INCOMPLETE ____

GUIDELINE: SATISFIED ___ PARTIALLY SATISFIED X NOT SATISFIED ___

DIRECT RVW TIME = START DATE: END DATE:

REVIEWED BY: Roger Gardner

TITLE: Toxicologist

ORG: TOX/Section 6

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SIGNATURE: Roger Gardner DATE: 8-28-86

APPROVED BY: Jane Harris, Ph. D.

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1. CONCLUSIONS: Diets containing 0, 1250, 2500, or 5000 ppm metalaxyl were fed to male and female mice for up to 90 days. Subsequently, some of the test animals were given control diets for an additional 30 days in a recovery phase of the experiment. There were minimal liver effects at the 2500 and 5000 ppm levels in males (changes in glycogen content, fat vacuolization, and hypertrophy in hepatocytes which were not observed after three months of treatment). Liver weights were elevated in all treated females after 90 days on the test diets, but no histopathological effects were reported for the liver of those animals. Absolute liver weights for test groups in the recovery phase of the study were comparable with those of the control group at termination. Since many of the effects are reversible during treatment of the male mice, and since liver weights in treated mice were comparable to those of untreated mice after 30 days without metalaxyl, the effects observed in the range finding study should be considered physiological or pharmacological.

Core classification: Supplementary. The study was a range finding experiment.

2. BACKGROUND

The study reviewed below was conducted to support the conclusion that a maximum tolerated dose (MTD) was achieved in a chronic toxicity study in mice. Dose levels in the chronic mouse feeding study were 50, 250, and 1250 ppm. A letter dated July 24, 1985 that accompanied the range finding study report stated that results from the 32-day interim sacrifice suggested that an MTD was achieved in the chronic study.

3. TEST SUBSTANCE: CGA 48988 Technical; purity unspecified.

4. MATERIALS AND METHODS

Test species: Male and female weanling CFLP (ICI strain 1 originated) mice were used. They weighed from 22 to 27 g, and were four to five weeks of age when received at the laboratory.

Experimental procedure: Mice were assigned to four groups each containing 85 individuals of each sex. Immediately prior to the start of the test, a fifth group containing 10 animals of each sex were selected for collection of baseline data. The mice in each test group were then given diets containing 0, 1250, 2500, or 5000 ppm test substance for up to 90 days.

All test animals were observed twice a day for signs of toxicity and mortality. Body weights for each animal were obtained on the day the test was started and at 5-day intervals through the end of the experiment. Food and water consumption were determined every fifth day for each animal.

Blood samples were taken from 10 male and 10 female mice before the test started to determine pre-test baseline data. These animals were then discarded from the study. During the feeding period, blood samples were collected from 10 animals of each sex from each group on days 5, 10, 20, 30, 60, and 90 and prior to termination on all survivors at the end of a 31-day treatment-free period. Blood was collected from the retro-orbital sinus, and the animals were fasted overnight prior to sampling.

4. MATERIALS AND METHODS (continued)

Observations of blood samples included:

Hematology

Hemoglobin concentration	Total leucocyte count	Mean cell volume
Packed cell volume	Differential leucocyte count	Mean cell hemoglobin
Reticulocyte count	Platelet count	Mean cell hemoglobin concentration
Erythrocyte count		

Blood Chemistry

Urea	Albumin
Alanine aminotransferase	Albumin/globulin ratio
Gamma glutamyl transpeptidase	Total bilirubin
Alkaline phosphatase	Lactate dehydrogenase
Total proteins	Cholesterol

Animals found dead or sacrificed during the study were necropsied; gross lesions were noted. The 20 animals used to gather baseline clinical pathology data before treatment was started were not necropsied; 10 animals of each sex in each group were sacrificed after days 5, 10, 16, 20, 32, or 60. Ten mice of each sex from each group were designated to be fed the control diet for a 31-day recovery period, but the remaining survivors were sacrificed after 90 days of treatment (approximately 15 mice per sex from each group). Those mice continued on control diets were sacrificed approximately 120 days after the experiment began.

Organs weighed at necropsy included:

Adrenal glands	Heart
Brain	Kidneys
Gonads	Liver

The following tissues and organs were preserved for microscopic examination:

Adrenals	Heart	Pituitary	Testes
Aorta	Ileum	Prostate	Thymus
Brain	Jejunum	Rectum	Thyroid (with parathyroids)
Cecum	Kidneys	Salivary gland	Tongue
Colon	Lungs (with (main stem bronchi)	Sciatic nerve	Trachea
Duodenum		Seminal vesicles	Urinary bladder
Epididymides		Skeletal muscle	Uterus
Esophagus	Lymph nodes	Skin	Vagina
Eyes	Mammary glands	Spinal cord	Abnormalities
Femur (and marrow)	Ovaries	Spleen	
	Pancreas	Stomach	

Statistical analyses: The protocol included in the supplementary report stated that the statistical methods are described in the Final Report which was not available for this review.

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4. REPORTED RESULTS

There were two males in the highest dose group that died after 31 to 35 days, and one each in the mid and low dose groups (after 16 to 20 days and 11 to 15 days, respectively). There were 5 deaths among female mice. One each in the control and high dose groups were observed after 66 to 70 days or 6 to 10 days, respectively, and 3 in the mid dose group (after 6 to 10 days, 31 to 35 days, and 61 to 65 days). The authors stated that most of the deaths resulted from blood sampling rather than the test substance.

According to the report, there were no effects on appearance or behavior of the animals. There were also no effects on hematology noted by the investigators.

The only clinical chemistry observation that was significantly affected was blood urea levels in male mice sacrificed after 90 days on the mid- and high-dose diets. Reported group mean urea levels were 72, 77, 88, and 87 mg% for the control, low, mid, and high dose groups, respectively. The mid and high dose group means were stated to be statistically significantly different from that for the control group at $p<0.05$ (Type of test used was not specified.).

Liver weight and liver-to-body weight ratios were increased in all treated animals when compared to those values for the untreated control groups after 60 and 90 days (see Table 1 below). However, only relative liver weights remained elevated in the high dose group females after the 31-day recovery period.

The report stated that there were no compound-related gross lesions observed in any of the mice necropsied in the study.

Most of the microscopic changes observed in the liver (only tissue reported in summary tables) were observed in males sacrificed after 33 days of the experiment. These changes are shown in Table 2 below. They were not observed in animals sacrificed at 90 days or in animals sacrificed after the recovery period.

The investigators summarized their observations of the livers of the test animals as follows:

> ...the initial changes within the periacinar hepatocyte, i. e., hypertrophy, increased fat, and decreased glycogen (feathery vacuolation) had virtually disappeared after three months of treatment...These These changes were confined to males receiving 2500 or 5000 ppm.

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4. REPORTED RESULTS (continued)

Table 1

Summary of group mean body weight (g), liver weight (g), and liver-to-body weight ratio (% body weight) for mice given test diets for 60 and 90 days or 90 days followed by a 31-day recovery period (reproduced from "Text Table 1" of the original report).

	Males				Females			
Observatin	0	1250	2500	5000	0	1250	2500	5000
				At 60 days				
Body weight	49.1	50.3	45.4	45.0	40.1	40.6	38.1	37.8
Liver weight	2.3	2.7*	2.6	3.0**	2.1	2.4	2.4	2.7**
Liver-to-body weight ratio	4.76	5.33*	5.76**	6.51**	5.21	6.00*	6.19**	7.02**
				At 90 days				
Body weight	58.0	58.6	56.1	53.0	42.7	43.5	41.8	41.1
Liver weight	3.3	3.9*	4.0*	4.3**	2.6	2.9*	3.3*	3.4**
Liver-to-body weight ratio	5.74	6.59**	7.07**	8.04**	6.05	6.71*	7.88**	8.34**
				90-day feeding + 31-day recovery				
Body weight	64.4	62.6	63.6	57.2	53.0	46.6	49.4	45.5*
Liver weight	3.6	3.2	3.7	3.4	2.8	2.7	2.6	2.7
Liver-to-body weight ratio	5.60	5.20	5.77	5.93	5.25	5.79	5.34	5.92*

*Statistically significantly different from controls, $p<0.05$.
**Statistically significantly different from controls, $p<0.01$.

Table 2

Summary of histological findings noted by the investigators in male mice treated for 33 days with metalaxyl (exerpted from Tables 7A through C of the original report)

Observation	0	1250	2500	5000
No. livers examined	10	10	10	10
Periacinar hepatocytic feathery vacuolation*	8	6	2	0
Periacinar hepatocytic fat	0	1	7	7
Periacinar hepatocytic hypertrophy	0	0	0	10

*Indicative of decreased glycogen.

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5. DISCUSSION

A statistically significant group mean body weight decrement (>10% below the control group mean) was not observed until the end of the recovery period and was limited to the 5000 ppm group females. Body weight gain or food efficiency may have been significantly decreased for that group early in the study, but there were no food consumption or body weight data presented other than that summarized in Table 1 above to provide a basis for evaluation of those effects.

The microscopically observed changes in glycogen content, hypertrophy and fat vacuolization in hepatocytes after 33 days of treatment (Table 2 above) indicates an effect early in the study. However, the absence of similar observations after 90 days suggests that the animals were capable of adapting to the effects of metalaxyl during treatment.

The reported elevation of blood urea may also suggest an effect, but no histopathology was included for the kidneys from which to determine the significance of the increase. Moreover, the findings of the long-term mouse feeding study with metalaxyl did not indicate histological effects consistent with the reported changes in blood urea in the 90-day study.

The results described above suggest that minimal reversible effects occur in the liver of male mice given diets containing 2500 or 5000 ppm metalaxyl for up to 90 days. Increased absolute liver weights were seen in all groups of female and male mice, but no histological changes were noted in these animals after 90 days of treatment. Under the conditions of the range finding study described above, all three doses caused minimal liver effects, and the results should be considered with those of other studies to determine their toxicological significance.