



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

DEC 31 1985

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Metalaxyl, Tox Chem. No. 275AA.

FROM: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769) *Reto Engler*

TO: Henry Jacoby, Product Manager #21
Herbicide Fungicide Branch
Registration Division

On June 21, 1985, a peer review committee was assembled to evaluate the toxicological data, in particular the long term studies on Metalaxyl. The members of the committee and principal reviewers are listed below.

A. Individuals in Attendance:

1. Peer Review Committee (Voting members): The signature indicates concurrence with this report unless otherwise stated.

Theodore M. Farber

William Burnam

Reto Engler

Louis Kasza

Bertram Litt

Jane Harris

Bernie Haberman

Donald Barnes

Robert Beliles

Theodore M. Farber
Wm. Burnam
Reto Engler
Louis Kasza
Bertram Litt
Jane C. Harris
Bernie Haberman 12/4/85
Donald H. Barnes
Robert O. Beliles 12/4/85

2. The data were presented to the committee by the following non-voting panel members; their signature indicates concurrence with the technical accuracy of this report.

Chad Sandusky

Robert Zendzian

Chad Sandusky 1/31/86
Robert Zendzian 1/14/85

B. Material Reviewed:

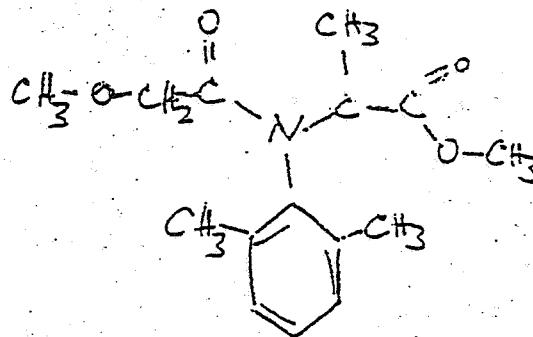
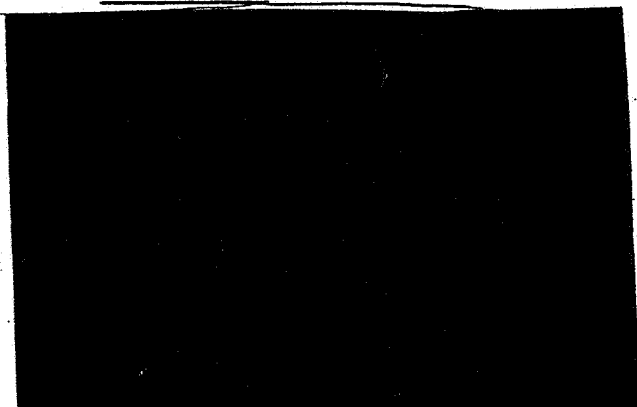
The material reviewed focused on the 2 year rat and mouse studies conducted at Life Science Research (LSR) in England. Substantial additional information on these studies was available, particularly histopathological reexamination by EPL, several evaluations of the histopathology by CAG (Dr. Haberman), GLP Laboratory Audit reports (Goldman et al.), and an independent evaluation of the laboratory audit (A. Gross) (total package attached].

C. Evaluation of Facts:

1. Structure Activity Relationships

The SAR was not specifically discussed at the committee meeting. After the meeting a cursory inquiry revealed that [REDACTED] was another fungicide structurally related to Metalaxyl. [REDACTED] has not shown an oncogenic response in a rat study; the mouse study is presently still under review.

Structure:



METALAXYL

Both compounds feature [REDACTED] as a structural component, however, the one-liners indicate no available metabolism studies, therefore information on biological activity based on SAR would be entirely speculative.

2. Short Term Tests

Two mutagenicity assays (Ames and dominant lethal in the mouse) are listed in the one-liners. Both tests were acceptable and negative. The tests were not further discussed by the committee; however, it should be noted that the testing is insufficient to fulfill the requirements of a mutagenicity battery.*

3. Other Toxicological Assays

Metalaxyl was not very toxic in several toxicity assays: the LD50 in rats is 669 mg/kg. In several subchronic exposure studies only minimal toxic effects were noted, for example, at 62 mg/kg/day (1250 ppm) in the rat 90 day study or at 31 mg/kg/day (1250 ppm) in the dog 90 day study or 25 mg/kg/day in a 6-month dog study. All these doses were the HDT. Reproduction was not affected at 1250 ppm (HDT) in the rat and in rat teratology studies as much 120 mg/kg were tolerated without adverse effects on the offspring. In another rat teratology study no maternal toxicity was observed at 20 mg/kg (HDT).

Integrating these results indicate that an MTD in these subchronic exposure studies has not been exceeded and very likely has not been reached.

4. Oncogenicity Studies:

a. Three 2-Year Rat Studies

The three major issues discussed concerned (1) parafollicular cell adenomas in the thyroid of female rats, (2) adrenal gland pheochromocytomas in male rats, and (3) whether the highest dose tested (1250 ppm) represents an MTD.

With respect to the thyroid tumors in female rats the group evaluated and discussed the reevaluation of slides at EPL and the review by Dr. Kasza (October 25, 1983). The findings are shown below:

* After the meeting the PM informed us that other mutagenicity tests were received by the Agency. These tests were all negative but not fully acceptable i.e., additional information was needed. See conclusion for request to finalize mutagenicity data submission.

FEMALES

Dose (ppm)	0	50	250	1250
<u>Original report</u> of C-Cell adenomas	2/80*	7/80*	10/79*	5/80*
<u>Reevaluation:</u>				
Adenomas	4/70	5/70	11/69	5/69
Carcinomas	1/70	2/70	1/69	0/69
Total	5/70	7/70	12/69	5/69
C-Cell Hyperplasia (Total)	20/70	22/70	29/69	25/69

* This number includes (ten) animals for interim sacrifice.

The committee agreed with the conclusion that thyroid tumors in the females are not compound related since:

- No progression to malignancy was observed
- No increased hyperplastic changes were observed
- No dose response was observed; the incidence of the middle dose is still statistically significant ($p < 0.05$), however the overall pattern (trend) shows no statistical significance ($p > 0.20$).
- The apparent effect reported earlier was mitigated by the re-evaluation of the thyroid slides.

According to the Laboratory Audit (A. Gross) the historical control rate reported earlier by the registrant may be less than adequate since they included terminal sacrifices as well as animals dying on test, and evaluation by several pathologists, hence the historical data were not used in reaching the above conclusion.

The issue concerning the alleged increase of adrenal gland pheochromocytomas due to compound administration was also re-assessed based on a re-evaluation of the microscopic slides by an outside pathologist at EPL. The overall evidence was reviewed by the CAG pathologist Dr. Haberman. The following table illustrates the findings:

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INCIDENCE OF ADRENAL GLAND PHEOCHROMOCYTOMAS IN MALE
CHARLES RIVER CD RATS FED METALAXYL FOR TWO YEARS

	0 ppm Control	50 ppm	250 ppm	1250 ppm
LSR (Pathology)	8/80 (10%)	6/80 (7.5%)	11/80 (13.8%)	16/80* (20%)
				Fisher Exact Test p = 0.058
				Test for linear trend p = 0.03
EPL (Pathology) (Re-reading)	5/80 (6.3%)	3/80 (3.8%)	6/79 (7.6%)	7/80 (8.8%)

The committee agreed that the re-evaluation reduced any suspicion of compound induced adrenal tumors effects in this study. Two experienced pathologists concurred with the re-evaluation.

The issue of the MTD was discussed at length. The essential part of this discussion centered on the question, whether a dose where "compound related effects" are noted can also be considered a "maximum tolerated dose." All members of the committee agreed that the first condition was met by this study i.e., compound related effects were shown, namely increased liver weights and periportal vacuolation of hepatocytes. The discussion thus focused on whether these effects represented an exhaustion of the animals tolerance i.e. MTD. The final consensus was that the minor, albeit compound related effects, were not consistent with an MTD. This conclusion is supported further by the above discussion on "Other Toxicological Assays." None of subchronic dosing regimens used a dose exceeding 1250 ppm and no significant toxic effects were noted, other than hepatocyte hypertrophy that were commensurate with an MTD or an exceeding of the MTD. [For example, if there were a subchronic rat study with a dose of 1500 ppm showing significant toxic effects, the choice of the high dose in the chronic study (1250 ppm) could be justified.]

It should also be noted that even the registrants consultant (Dr. Friess) could not come to an unequivocal conclusion that the MTD had been reached.

The registrant also cites a 28 day range-finding study conducted with 10 male and 10 female rats in which the compound was administered in increasing doses. At the highest exposure the rats received 100 mg/kg/day for 2 weeks, 300 mg/kg/day for 1 week and 600 mg/kg/day for an additional week. Even at this dosing regimen the toxic effects were not severe, i.e. no deaths, no effect on weight gain. Effects on the liver and testes were noted and the animals showed tremors for one day after the dose was raised to 600 mg/kg. This experiment also indicates that an exposure of 62 mg/kg/day (1250 ppm) is unlikely to represent an MTD.

b. 2-Year Mouse Study

Two major issues were discussed concerning the mouse oncogenicity assay: (1) the incidence of liver tumors in male mice, and (2) the issue of the MTD.

The situation concerning the liver tumors was very similar to those discussed above concerning tumors in rats; i.e. LSR had reported tumor incidences which appeared to show a compound related effect, whereas re-examination of the histopathology by EPL and the CAG pathologist pointed to a misdiagnosis by LSR. The original incidences and reevaluation are shown below.

Liver Tumors in Male Mice (CA/AD Combined)

Dose (ppm)	0	50	250	1250
Original report	14/60	22/60	17/60	23/60
EPL Re-examination	20/60	23/60	20/60	24/60

The committee concurred that the re-evaluation was reliable and did not show any compound related effects on the incidence of liver tumors in the mouse. In addition to the combined incidence shown above, the proportion of carcinomas did not increase in the treated groups.

The discussion concerning the MTD in this mouse study was along the same line as the one on the rat study. 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 (neither report has apparently been submitted to the Agency in unabridged form). The registrants own conclusion is quoted below:

"It is concluded that evidence of toxic effects was apparent at dietary levels of 5000 ppm (namely inferior bodyweight gain, disturbances in the chemical composition of the blood and higher liver weight) and 2500 ppm (disturbances in the chemical composition of the blood and higher liver weight). A possible effect (higher liver weight only after 10 or 20 and 32 days of treatment) was also evident at a dietary level of 1250 ppm."

This indicates that 1250 ppm chosen for the long-term study represented a minimal rather than a maximal dose to be tolerated by mice.

D. Conclusion:

The committee concluded that the experimental data of both the mouse and rat oncogenicity assays did not show an oncogenic response. The marginal effects originally reported by LSR were refuted by independent review of data by EPL and CAG pathologists. The mutagenicity assays at hand as well as the reviews (Stanley Gross, undated) submitted by the PM after the meeting support this conclusion. The committee noted that a full battery of mutagenicity assays should be evaluated as soon as possible. For this purpose the registrant should clarify the issues associated with the review of the studies and he should also re-evaluate the mutagenicity battery for conformity with present day requirements, covering the major categories of mutagenicity.

On the other hand, the committee concluded that for both the rat and mouse oncogenicity studies an MTD had not been reached although treatment-related changes in liver histology were noted at the highest dose tested. This conclusion is supported by the evaluation of several subchronic dosing regimens which showed only minimal toxic effects on the liver at 1250 ppm in rats and mice. A final determination concerning the impact of this deficiency of the two studies will be done after the genotoxicity studies have been fully evaluated.