10-24-86

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

005475

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: MANCOZEB - Toxicology Chapter of the Registration Standard

FROM: Irving Mauer, Ph.D. 10/24/86
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TO: Esther Saito
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THRU: Jane E. Harris, Ph.D.
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and

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and

10/24/86
Theodore M. Farber, Ph.D., Chief
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Please find attached the Toxicology Chapter for the Mancozeb Registracion Standard, consisting of the following sections:

A. Toxicology Executive Summary;

B. Toxicology Profile (FIFRA Subdivision F, 40 CFR 158.135, Series 81 through 85);

C. Summary of Data Gaps;

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D. Tolerance and Tolerance Reassessment;

E. Toxicology Issues;

F. Data Requirements Summary Tables (A, B);

G. Bibliography;

H. One-Liners; and

I. Data Evaluation Reports.

Attachments

cc: Amy S. Rispin (FEB/SIS)

Caswell No. 913A

EPA Chem. No. 014504

TB Project No. 28

005425

MANCOZEB

REGISTRATION STANDARD

TOXICOLOGY CHAPTER

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Toxicology Branch
Hazard Evaluation Division (TS-769C)

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A. TOXICOLOGY

EXECUTIVE SUMMARY

Technical mancozeb is relatively nontoxic on an acute basis, as demonstrated in adequate acute toxicity studies (TOXICITY CATEGORIES III to IV).

Adequate subchronic studies (90-day) indicate mancozeb induces minimal effects on the rat thyroid (the assumed principal target organ) by the dietary route at 250 ppm (= 18.75 mg/kg/day), and in the dog at 5000 ppm (= 125 mg/kg/day), generating no-effect levels of 125 ppm (= 9.375 mg/kg/day) and 1000 ppm (= 25 mg/kg/day), respectively. However, lower nonthyroid effect levels were also found in these feeding studies, namely, renal tubular degeneration at 125 ppm in male rats (NOEL = 60 ppm, or 4.5 mg/kg/day), and cortical lymphoid depletion in the thymus as well as prostatic hypogenesis in dogs at 1000 ppm (NOEL = 100 ppm, or approximately 2.5 to 3.0 mg/kg/day). Adequate data from long-term (2-year) studies for chronic effects/oncogenic potential (to satisfy current regulatory requirements) are not available, in order to ascertain the persistence or toxicological consequences of these effects (both thyroid and nonthyroid). However, older (1965) but inadequate chronic studies (judged SUPPLEMENTARY DATA) have reported decreased I^{131} uptake and thyroid hyperplasia in dogs fed 1000 ppm for 2 years, also generating a NOEL of 100 ppm.

A 90-day inhalation study with mancozeb is also available which indicates the presence of granular pigment in renal tubules of rats exposed to a nominal concentration of 80 mg/m^3 (corresponding to a respirable concentration of 36 mg/m^3), with a NOEL of 20 mg/m^3 nominal (9 mg/m^3 respirable), which is a dose lower than that at which reduction in T4 levels and hyperplasia of thyroid follicular epithelium were recorded (i.e., 320 mg/m^3 nominal, or 144 mg/m^3 respirable). Data from comparable dermal studies are not available to define the risk for this route of human exposure; adequate data from percutaneous absorption (dermal penetration) testing are still required.

Adequate studies defining reproductive effects are also not available; however, an older (1965) supplementary study reported reduction in fertility at the highest dose tested, 1000 ppm (NOEL = 100 ppm, or approximately 5 mg/kg/day), but no other parental, reproductive, or fetal effects at this level. Thyroid effects (increased relative organ weight, lower PBI and hyperplasia) were recorded after 3 months feeding diets containing 1000 ppm mancozeb to the F_1b (F_2a) generation of this study, but no such effects at 300 ppm (approximate intake, 15 mg/kg/day). Adequate data from a rat teratology study reported fetal and teratologic effects only at doses higher than that causing maternal toxicity (A/D ratio = 0.25); an older (1968) inadequate rabbit study (SUPPLEMENTARY DATA, which the company is replacing), recorded only slight decreases in maternal weight at the higher (of two) doses tested, 250 mg/kg/day, but no other clinical, fetal or teratogenic effects (provisional A/D ratio = 0.1).

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Except for direct (primary) DNA repair (only one inconclusive study, reporting presumptively positive UDS in rat hepatocytes, which should be repeated), adequate genotoxicity studies record mancozeb to be negative for both bacterial and mammalian gene mutations, negative for in vivo chromosome damage (but positive in one in vitro sister-chromatid exchange assay), and negative for in vitro mammalian cell transformation. Requirements for promotion assays were not satisfied by the unacceptable studies submitted for both mancozeb and ETU.

Adequate metabolism studies in rats with radioactively labeled (C-14) mancozeb demonstrate that the EBDC is rapidly absorbed from the gut and excreted in approximately equal portions in urine and feces (and to a much lesser extent in bile), accumulating in a number of major organs (with the highest accumulation in the thyroid). Residue analysis for ETU detected measurable amounts only during the 24 hours following a 100 mg/kg dose, averaging 1 ppm in thyroid and 0.66 ppm in liver.

Since there are no adequate chronic studies to support the tolerances previously established for mancozeb, only the subchronic studies recently submitted (and judged fully satisfactory by current regulatory Guidelines) can be used as reference doses. Thus, a PADI has been drawn from a NOEL of 3 mg/kg/day in the 90-day dietary dog study, which thus occupies 94% of the current TMRC (applying a 1000 SF). This is supported by an older (CORE SUPPLEMENTARY) study in dogs generating a comparable NOEL (= 100 ppm) based on thyroid effects at the next highest dose level (1000 ppm).

In addition to the necessity for tolerance reassessment, several other major issues from this evaluation of available data on technical mancozeb concern the uncertainties regarding:

1. Whether toxicologically effective concentrations of ETU (an acknowledged goitrogen, teratogen, and oncogen) can be generated from human exposure to this EBDC via the three major routes (dietary, inhalation, and dermal).

2. Whether derivatives other than ETU contribute to risk for nonthyroid human organ systems, as demonstrated by subchronic animal studies to date, and by the apparent species-specific sensitivities so far recorded in older, less-than-adequate chronic studies.

3. Whether one, or several, mechanism(s) of action need be defined to explain the variable results of toxicological assays to date, specifically the effects seen in a number of nonthyroid organ systems and tissues. These concerns can only be resolved by the submission of fully adequate long-term/lifetime studies.

B. TOXICOLOGY PROFILE

[According to the Federal Insecticide, Fungicide, and Rodenticide Act Pesticide Assessment Guidelines, Subdivision F, 40 CFR 158.135, Series 81 through 85]

Series 81: Acute Toxicity and Irritation Studies

Except for dermal sensitization, adequate studies exist to define the acute toxicity of both mancozeb technical (typically 80% ai +) and various forms of manufacturing-use products (containing approximately 35% ai). The majority of these assays (summarized below and in One-Liners, Section H) indicate this EBDC presents little or no acute hazard potential (Toxicity Category III or IV); only one 35% MP formulation presented results which could be considered moderately toxic (Toxicity Category II) for acute inhalation and eye irritation (submitted under Accession Nos. 244505 and 244298.

No further studies are required at this time for acute oral, acute dermal, acute inhalation, primary eye, and primary skin irritation. However, the absence of any testing for dermal sensitization makes this type of study required for both the TGAI and current MP formulations.

Series 82: Subchronic Testing

In response to the Data Call-In Notice of October 19, 1984, recently submitted 90-day studies have been found to satisfy data requirements for subchronic testing in the rat and dog by the dietary route, and in the rat by inhalation.

82-1: Oral (Rodent, Nonrodent) Studies

When fed to Sprague-Dawley rats at levels of 0, 30, 60, 125, 250 and 1000 ppm (a seventh group received 250 ppm ETU), no gross clinical effects or histological changes in the thyroid were reported at levels of 250 ppm or below; depressed body weight and changes in hormone levels accompanied by diffused hyperplasia of thyroid follicular epithelium were recorded in 1000 ppm males and females (MRID IMM203). However, slight but significant depression of T4 levels also occurred in females fed 250 ppm; hence, the NOEL for thyroid effects is 125 ppm (= calculated intakes of 7.9 and 9.2 mg/kg/day for males and females, respectively). Residue analysis for ETU in mancozeb-treated animals revealed none in blood, but dose-related increases in urine (from 0.3 ppm at the LDT to 10 ppm at the HDT, i.e., a 1% conversion), and thyroid (< 4 ppm at 30 ppm mancozeb to 25 ppm at 1000 ppm mancozeb, i.e., at least a 2.5 percent conversion). Although discounted by the authors as being compound-related, deposits of yellow-brown pigment were present in the lumen of renal cortical tubules at all doses above 60 ppm (both sexes), accompanied by an increased incidence of multifocal cortical tubular degeneration in 125 ppm mancozeb males. The authors suggested these granules were deposits of the excretory metabolite, ethylenebisisothiocyanate sulfide, or EBIS (none were found in ETU animals). Based upon these histopathological changes in kidneys at levels of 125 ppm

SERIES	TEST MATERIALS:					
	TECHNICAL (80% ai +)			MP (35-36% ai)		
		TOX.CAT.	MRID	LD^{50}	TOX.CAT.	MRID
81-1: Oral (mg/kg)	LD_{50}: 4500 > 5000 > 5000	III IV IV	047146 142522 093926	LD_{50}: > 5000 > 5000 > 5000	IV IV IV	IMMZ01 097182 126511
81-2: Dermal (mg/kg)	LD_{50}: > 10000 > 5000 > 5000	III III III	047146 142522 093927	LD_{50}: > 2000 > 5000 > 5000	III III III	IMMZ01 097182 126511
81-3: Inhalation (mg/L)	LC_{50}: > 6.85 > 5.14	IV IV	047146 093928/ 145996	LC_{50}: > 0.35	II	IMMZ02
81-4: Eye irritation	PIS: 2.3	III	42522	PIS - 0 0.1	II IV III	IMMZ01 097182 126511
81-5: Skin irritation	PIS: 0 0.2 0.5	IV IV IV	047146 142522 093927	PIS - 0.4/0.8 0	III IV IV	IMMZ01 097182 126511

and above, the systemic NOEL for mancozeb is set at 60 ppm, equivalent to calculated intakes of 3.5 mg/kg/day in males and 4.4 mg/kg/day in females. Finally, compound-related effects in 250 ppm ETU-treated animals were generally comparable to those observed at 1000 ppm mancozeb.

A comparable subchronic study was performed in beagle dogs fed diets for 13 weeks containing 0, 10, 100, 1000, and 5000 ppm mancozeb (but there was no ETU group)(MRID IMMZ04). Significant clinically toxic effects were noted only at the two highest dose levels, namely, dose-related anorexia and dehydration (two males and one female at 5000 ppm had to be sacrificed *in extremis* during the course of the study), decreased food consumption, and body weight loss (especially in females). Reduction in red cell mass and related values were also noted in high-dose animals of both sexes, as well as: (i) histomorphological evidence of hypothyroidism; (ii) pallor in the zona fasciculata of adrenals; (iii) hypoplasia of gonads and sex organs (especially prostatic hypogenesis in males); and (iv) cortical lymphoid depletion in the thymus. Since some 1000 ppm animals also manifested these effects, the overall systemic NOEL is determined to be 100 ppm, equivalent to calculated intakes of approximately 3.0 mg/kg/day for males and 3.4 mg/kg/day for females. Based upon thyroid effects only at the HDT (follicular cell hyperplasia, decreased T3/T4, and related hypercholesterolemia and hyperbilirubinemia), a NOEL for this organ may be set at 1000 ppm (29 mg/kg/day for both sexes).

A 90-day dietary study in CD-1 mice has been submitted in which animals were fed mancozeb at levels of 0, 10, 100, 1000, and 10,000 ppm (MRID IMMZ06, Accession No. 259888, Doc. #005038). Increased incidences of thyroid follicular cell hyperplasia/hypertrophy and decreased hepatic aminopyrine N-demethylase activity were reported at 1000 ppm and higher, thus providing a provisional NOEL of 100 ppm, equivalent to a calculated intake of approximately 18 mg/kg/day, and 22 mg/kg/day for females.

No further subchronic oral studies in rodents or nonrodents are required at this time.

82-2/82-3: Dermal (21-day/90-day) Studies

No adequate subchronic dermal studies were available for review. One older (1965) subacute schedule involved the daily application of 5000 mg/kg of an uncharacterized technical (undiluted "Zimaneb," stated to contain 80% ai) for 5 days to the clipped abdomens of four male and four female rabbits (half of whom were abraded), without clinical effects (MRID 047146).

However, adequate 21-day dermal data are required to determine both cutaneous and systemic effects of longer applications of technical mancozeb.

82-4: Inhalation (90-day) Studies

A series of adequate studies involving repeat schedules of rats exposed to mancozeb technical has recently been submitted (MRID [illegible]).

Following a 2-week range-finding study designed to determine exposure levels and mode of exposure, Sprague-Dawley rats of both sexes ([illegible] sex/group) were exposed "nose-only" to target (nominal) aerosol concentrations of 0, 20, 80, and 320 mg/m^3 (6 hrs/day, 5 days/week for a projected 13 weeks), corresponding to respirable concentrations of 0, 8, 36, and 144 mg/m^3, respectively. After 4 weeks of exposure significant reductions were noted for high-dose males; additionally, after 13 weeks' treatment, reduction in T4 levels in high-dose females were recorded, accompanied by hyperplasia of follicular epithelium. Residue tissue analysis at 13 weeks (blood, urine, thyroids) revealed increasing (mancozeb dose-related), concentrations of ETU in urine and thyroid, especially in females. The presence of yellow-brown granular pigment in renal tubules of both high-dose and mid-dose males and females was noted. Based upon these renal inclusions (which may progress to a form 6 of kidney urolithiasis), the systemic NOEL is determined to be the LDT, nominally 20 mg/m^3 (= 8 mg/m^3 respirable), while the thyroid NOEL is 80 mg/m^3 nominal (= 36 mg/m^3 respirable). No further inhalation studies are required at this time.

Series 83: Chronic and Long-Term Studies

[illegible] adequate studies defining long-term effects of [illegible]. A number of older studies, however, [illegible] some useful information (judged Supplementary-Data).

Series 83-1: Chronic Toxicity Studies

In a 1965 report, weanling Wistar albino rats (25/sex/group) were fed Dithane M-45 technical (86% ai) at levels of 0, 25, 50, 100, and 1000 ppm in what was projected to be a 2-year study (MRID 00077[illegible]). During the first 6 months, treated males and females were mated twice to provide animals for a three-generation reproduction study (discussed below), then treatment was continued following these matings. The study, however, had to be terminated at 90 weeks (21 months) because of excessive mortality in all groups. No clinical effects or gross lesions were described in the final report which would have contributed relevant information preventing completion of the projected schedule for this study; the only significant finding reported was thyroid hyperplasia in high-dose (1000 ppm) animals of both sexes, as well as in a

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few males at each of the lower doses (including the LDT). Since neither individual animal nor clinical chemistry data were provided, and insufficient numbers of animals were examined histopathologically, this study is classified CORE-SUPPLEMENTARY.

During the same period, the same laboratory also conducted a 2-year study in beagle dogs (4/sex/group) fed a Mazola/cod liver oil mixture of mancozeb at levels of 0, 25, 100, and 1000 ppm (MRID 080514). No clinical toxicity was reported at any dose, body weight gains and food consumption were unaffected by treatment, and all hematological and clinical chemistry values were normal, except for consistently lower I^{131} uptakes recorded in high-dose animals. Only summary histopathological data were available at the time of this review (included as Table 17 of the Final Report), which indicate focal hyperplasia in the thyroid of one mid-dose (100 ppm) male, and Grade-1 hyperplasia in two high-dose (1000 ppm) animals (one male, one female) both of which had lower than control I^{131} values.

Although no individual animal data were included in the final report of this study (in addition to other deficiencies, allowing only grading of CORE SUPPLEMENTARY according to current Guidelines), this study is otherwise minimally adequate in all other respects. Thus, based upon these thyroid effects at 1000 ppm, a provisional NOEL of 100 ppm can be substantiated (equivalent to an actual intake of 2.5 mg/kg/day), consistent with the systemic NOEL of 3 mg/kg/day demonstrated in the fully adequate 90-day dog study discussed above.

An adequate rat chronic study and a new dog chronic study (or further information supporting the study on file) are required.

83-2: Oncogenicity Studies

No studies assessing oncogenicity in rodents were available for review.

Oncogenicity studies in both rats and mice are required for continued registration of mancozeb.

83-3: Teratology Studies

An adequate rat teratology study has been submitted (MRID 093929), and the following developmental toxicity parameters defined:

Maternal toxicity:	NOEL =	32 mg/kg/day
	LEL =	128 mg/kg/day (decreased body weight)
Fetal toxicity:	NOEL =	128 mg/kg/day
	LEL =	512 mg/kg/day (increased resorptions)

Teratogenic effect: NOEL = 128 mg/kg/day
LEL = 512 mg/kg/day (dilated ventricles, spinal cord hemorrhage, delayed/ incomplete ossification of skull skull and ribs)

A/D Ratio = $\frac{32}{128} = 0.25$

In the same study, 50 mg/kg/day ETU decreased fetal weight, induced kidney and CNS lesions, and decreased ossification of skull and vertebrae.

An older (968) rabbit study (MRID IMM207) employed three groups of New Zealand White female rabbits orally intubated at 0 (corn oil), 25, and 250 mg/kg/day (10 rabbits each), and reported only a slight decrease in maternal weight at the HDT, but no maternal or fetotoxicity, and no gross or histological developmental abnormalities. The teratology phase, however, had an insufficient number of animals per dose group and has been classified CORE-SUPPLEMENTARY Data; hence, a new rabbit study is required.

83-4: Reproduction

An adequate study assessing reproductive effects of manecozeb over at least two generations is not available. However, four of the five groups of young Wistar rats which were being fed mancozeb for a projected 2-year period (MRID 080713) at levels of 0, 25, 100, and 1000 ppm were mated twice (the 50 ppm group was not mated) to provide the F_{1a} and F_{1b} for a projected three-generation reproduction study (2 litters/ generation, 20 matings each) (MRID 080715). Although individual animal data were not available, and only pooled summary results presented in the Final Report, no apparent clinical clinical or reproductive effects were reported at any level, except for reduction of fertility (percent mated females that became pregnant) at the HDT. No fetal values were reportedly affected, and no histopathological lesions attributable to treatment. Hence, provisional reproductive toxicity parameters may be set as follows:

Parental clinical	NOEL > 1000 ppm (= 50 mg/kg/day)
Reproductive	NOEL = 100 ppm (= 5 mg/kg/day)
	LEL = 1000 ppm (decreased fertility)
Fetal	NOEL > 1000 ppm

In an addendum to this series of reports (MRID 080713/080715), thyroid effects were examined in 15 males and 15 females from the F_1b (F_2a) generation after 3 months on diets containing 100 and 1000 ppm mancozeb, and in an additional intermediate dose group treated at 300 ppm mancozeb for 3 months. As positive controls, a further group of 10 F_2a weanlings of each sex were were fed 300 ppm of the known goitrogen, propylthiourea (PTU) for the same period of time, and additional F_2a weanlings originally on control diets were placed on 1333 ppm PTU for the last 5 weeks of the same 3-month period. Only PTU-treated animals, and to a much lesser extent those on 1000 ppm mancozeb manifested thyroid dysfunction, as evidenced by increased relative organ weight, lower PBI, and hyperplasia; no such thyroid effects were observed in either 300 ppm or 100 ppm mancozeb animals (respective intakes = 15 and 5 mg/kg/day). Further, only PTU-treated rats showed markedly lower I^{131} uptakes but, despite the presence of severe thyroid hyperplasia, no changes in PBI values.

A new reproduction study is required, failing the registrant providing data from the older study, as indicated above.

Series 84: Mutagenicity

Except for direct (primary) DNA damage/repair (84-2.3), requirements for the basic set of mutagenicity assays for the other categories of genetic effects have been satisfied by the following ACCEPTABLE data (MRID IMMZ08).

Series	Assay Type	Test System	Reported Result
84-2.1	Gene mutation	Ames (Rat S-9)	Negative
	Gene mutation	Ames (Mouse S-9)	Negative
	Gene mutation	HMA (Mouse)	Negative
	Gene mutation	CHO cells	Negative
84-2.2	Chromosome damage	Rat BM	Negative
	Chromosome damage	CHO/SCE	Positive
84-2.4	Other mechanisms	Transformation	Negative

Briefly, mancozeb is negative for gene mutation in both bacterial (Salmonella-Ames Tests and host-mediated assays) and in vitro mammalian cell (Chinese hamster ovary cells, testing at the HGPRT locus) systems, for chromosome damage in vivo (rat bone marrow cells), and in one mammalian cell transformation assay (mouse C3H 10T 1/2 cell cultures), but positive for sister-chromatid exchanges in CHO cells in vitro.

An assay for unscheduled DNA synthesis (UDS) in primary rat hepatocyte (HPC) cultures was classified inconclusive and

must be repeated, because of uncertainties in the validity of the presumptive positive result generated (e.g., the apparent increase in net nuclear grain counts was compromised by a rather high background, presumably due to cytotoxicity).

Further, the Data Call-In Notice of January 17, 1983 concluding the Special Review for the EBDC required the submission of adequate data for in vitro mammalian cell transformation/promotion. The negative studies submitted for this requirement (for both mancozeb as well as ETU) were judged UNACCEPTABLE because the assays were conducted at only one dose, which may have been insufficient to conclude the test materials were not in vitro promoters.

Series 85: Special Studies

85-1: Metabolism

Also in response to the Data Call-In Notice of January 17, 1983, an adequate metabolism study in rats has been submitted (MRID IMMZ09). As reported in 3 parts, groups of 3 to 5 young adult (6-weeks old) Sprague-Dawley rats of both sexes were given single oral doses of either 1.5 (low-dose) or 100 (high-dose) mg/kg ^{14}C-mancozeb (in one group, following 2-week dietary administration of 15 ppm unlabeled mancozeb), and total radioactivity and excretion of metabolites as well as residues of both the EBDC and ETU in selected tissues, determined at selected time periods up to 96 hours. Mancozeb appears to be rapidly absorbed from the gastrointestinal tract, distributed to target organs (highest accumulation of radioactivity found in thyroid within 24 hours), and excreted (by non-linear kinetics between low- and high-dose) almost totally by 96 hours. Residues of ETU in the thyroid averaged 1 ppm during the 24 hour period following the 100 mg/kg mancozeb dose (none were detected in low-dose animals), but was below the level of detection (0.012 ug/sample) thereafter, liver ETU residues averaged 0.5 ppm 6 hours after 1.5 mg/kg mancozeb, and 0.66 during the 24 hours following 100 mg/kg, and undetectable (limit = 0.014 ppm) thereafter.

85-2: Domestic Animal Safety

In the only available study (MRID 129288, dated October 1970), mancozeb was fed to male and female broiler chickens (of unstated strain) for 8 weeks at levels of 0, 0.02%, 0.10%, and 0.20% (0, 200, 1000, and 2000 ppm, equivalent to approximate intakes of 0, 30, 150, and 300 mg/kg/day). Deaths, severe dose-related clinical toxicity, less body weight gain, and increases in relative organ weights for both thyroids and livers were reported at both mid and high levels, but selected hematological values were comparable in all groups. Follicular enlargement, colloid accumulation, and fused or cystic irregular follicles were pronounced in high-dose birds, but were also recorded in a few birds fed 0.1% mancozeb diets. Hence a provisional NOEL

of 0.02% (200 ppm) may be set for this study. Since no individual animal data were provided, and no reporting on neural histology, however, this study is considered SUPPLEMENTARY DATA.

85-3: Dermal (Percutaneous) Absorption/Penetration

Also in response to the Data Call-In Notice of January 17, 1983, the registrant submitted a dermal penetration study in which 10 mg of commercial Dithane M-45 (8.3% ai, containg 0.04% ETU) was applied to a 20-cm^2 shaved area of the dorsum of adult female Sprague-Dawley rats, and secured in place under an elastic bandage for 6 hours (MRID 127950). Calculation of residues in urine and feces collected over the 6-hour exposure time and 18 hours later (following termination of treatment), permit a general value of 1% absorption to be utilized. Since only one dose was employed, however, the maximum absorption rate cannot be determined.

A study using technical mancozeb must be submitted to satisfy regulatory requirements.

Human Studies

Two reports are available monitoring potential effects of human mancozeb exposure.

In one undated survey of unstated source (cited as Document #003244 in a memorandum: Coberly to Nash, March 6, 1968), 54 manufacturing plant workers were subjected to physical examinations, blood study, and urinalysis over a period of 8 months (MRID 130638). This group continued in good physical health over this period of time without any reported thyroid, respiratory, gastrointestinal, or skin manifestations that could be attributed to mancozeb. These men were presumably exposed to greater concentrations of mancozeb than would be encountered by agricultural workers handling and applying the same material, although no exposure levels accompanied this report.

In the second ("Report of the Analysis of Dithane M-45 and ETU . . . in Urine Samples from Applicators and Mixer-Loaders . . . in Ohio" by R.O. Mumma et al., Penn State University, February 17, 1983, as TB Document 004726), ETU, but not the parent mancozeb, was detected in the urine of aerial applicators (pilots) at a level of 0.2 ppm (MRID 130638). These trials were conducted in Michigan and Minnesota; the urine of mixer-loaders was negative. All trials in other states were negative. These results suggest that agricultural use of mancozeb, and probably other EBDC's, results in a least some applicator exposure to the parent compound and the common metabolite, ETU. Quantification of this exposure, however, was also not possible from these data.

C. DATA GAPS

Based upon review and evaluation of available data, EPA does not have adequate data for the following which are required to be submitted under FIFRA 3(c)(2)(B):

For the TGAI:	Dermal sensitization	(81-6)
	Subchronic (21-day) Dermal Study	(82-2)
	Subchronic (90-day) Dermal Study	(82-3)
	Chronic Toxicity Study - Rodent	(83-1)
	Chronic Toxicity Study - Nonrodent	(83-1)
	Oncogenicity Study - Rat	(83-2)
	Oncogenicity Study - Mouse	(83-2)
	Teratology Study - Rabbit	(83-3)
	Reproduction Study - Rat	(83-4)
	Mutagenicity - DNA damage/repair	(84-2.3)
	Mutagenicity - Promotion	(84-2.4)
	Dermal (Percutaneous) Absorption	(85-3)
For Current MP's:	Dermal sensitization	(81-6)

D. TOLERANCES AND TOLERANCE REASSESSMENT

D. TOLERANCE REASSESSMENT

Mancezob is registered for use as a seed treatment for cotton, and as a fungicide for diseases affecting a wide variety of ground and tree fruits, field and garden vegetables, nuts, and cereal grains. Tolerances ranging from 0.1 to 10 ppm have been set for these crop groups and 0.5 ppm as secondary limits for kidney and liver (see attached), providing a TMRC (1.5 kg diet, 60 kg bwt) of 0.028296 mg/kg/day. Although there are no adequate chronic studies to support a firm NOEL in order to determine an ADI (especially in the rat), a PADI can be drawn drawn from the lowest NOEL's found in both the recently submitted subchronic dietary dog study (MRID IMMZ04, discussed above; and memorandum: Mauer to Farmer, dated July 20, 1986), as well as the older chronic dog study (MRID 080714), i.e., in the range of 2.5 to 3 mg/kg/day. Applying a thousandfold safety factor to the NOEL derived from the subchronic dog study a PADI of 0.003 mg/kg/day can be calculated. The current occupancy by the TMRC of this PADI exceeds 940 percent (0.028296 0.0030 = 9.432).

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TOXICOLOGY BRANCH ADI PRINTOUT Date: 06/11/86

THE FOLLOWING INFORMATION WAS SUPPLIED BY THE USER:

Chemical name: MANCOZEB = MG/KG
Caswell #915A Safety factor =
CFR No.

RESIDUE CONTRIBUTION OF PUBLISHED TOLERANCES

	CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY
2	Apples	7.000		2.53	0.265650000
5	Asparagus	0.100		0.14	0.000210000
7	Bananas	0.500		1.42	0.010650000
8	Barley	5.000		0.03	0.002250000
24	Carrots	2.000		0.48	0.014400000
28	Celery	5.000		0.29	0.021750000
39	Corn, pop	0.500		0.08	0.000600000
40	Corn, sweet	0.500		1.43	0.010725000
41	Cottonseed (oil)	0.500		0.15	0.001125000
42	Crabapples	10.000		0.03	0.004500000
44	Cranberries	7.000		0.03	0.003150000
46	Cucumbers, including pickles	4.000		0.73	0.043800000
66	Grapes, including raisins	7.000		0.49	0.051450000
68	Corn, grain (field corn)	0.100		1.00	0.001500000
92	Melons	4.000		2.00	0.120000000
102	Oats	5.000		0.36	0.027000000
106	Onions, dry bulb	0.500		0.72	0.005400000
109	Papayas	0.000		0.03	0.000000000
115	Peanuts	0.500		0.36	0.002700000
116	Pears	10.000		0.26	0.039000000
132	Quinces	10.000		0.03	0.004500000
140	Rye	5.000		0.03	0.002250000
154	Sugar, cane and beet	2.000		3.64	0.109200000
155	Summer squash	4.000		0.03	0.001800000
163	Tomatoes	4.000		2.87	0.172200000
170	Wheat	5.000		10.36	0.777000000
203	Kidney	0.500		0.03	0.000225000
211	Liver	0.500		0.03	0.000225000
214	FENNEL	10.000		0.03	0.004500000

TMRC
0.028296 mg/kg/day (60kg BW, 1.5kg diet) %
****

RESIDUE CONTRIBUTION OF TOX-APPROVED TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY

No tox-approved tolerances are listed in the file.

TMRC %
0.028296 mg/kg/day (60kg BW, 1.5kg diet) **********

RESIDUE CONTRIBUTION OF NEW (PENDING) TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY

No new tolerances are listed in the file.

TMRC %
0.028296 mg/kg/day (60kg BW, 1.5kg diet) **********

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§ 180.176 Coordination product of zinc ion and maneb; tolerances for residues.

Tolerances for residues of a fungicide which is a coordination product of zinc ion and maneb (manganous ethylene-bisdithiocarbamate) containing 20 percent manganese, 2.5 percent zinc, and 77.5 percent ethylene-bisdithiocarbamate (the whole product calculated as zinc ethylenebisdithiocarbamate), are established as follows:

65 parts per million in or on peanut vine hay, sugarbeet tops.

25 parts per million in or on the straws of barley, oats, rye, wheat.

10 parts per million in or on crabapples, fennel, pears, quinces.

10 parts per million in or on the whole fruit of papayas, with no residue present in the edible pulp after the peel is removed and discarded.

7 parts per million in or on apples, cranberries, and grapes.

5 parts per million in or on celery; corn fodder and forage; and the grains of barley, oats, rye, and wheat.

4 parts per million in or on bananas, preharvest use only, of which not more than 0.5 part per million shall be in the pulp after peel is removed and discarded.

4 parts per million in or on cucumbers, melons, summer squash, and tomatoes.

2 parts per million in or on carrots, sugar beets.

0.5 part per million in or on popcorn grain, fresh corn including sweet corn (kernels plus cob with husk removed), cottonseed, kidney, liver, onions (dry bulb), and peanuts.

0.1 part per million (negligible residue) in or on asparagus.

0.1 part per million in or on corn grain (except popcorn grain).

[36 FR 22840, Nov. 25, 1971, as amended at 37 FR 14170, Aug. 11, 1972; 37 FR 24118, Nov. 4, 1972]

§ 180.177 Ronnel; tolerances for residues.

Tolerances for residues of the insecticide ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate), including its 2,4,5-trichlorophenol containing metabolites, in or on raw agricultural commodities are established as follows:

10 parts per million in fat of cattle, goats, and sheep.

4 parts per million in meat and meat byproducts of cattle, goats, and sheep.

3 parts per million in fat of hogs.

2 parts per million in meat and meat byproducts of hogs.

1.25 parts per million in milk fat (reflecting negligible residues in whole milk).

0.5 part per million on bananas (of which residue, zero shall be in the pulp after peel is removed and discarded).

0.03 part per million in eggs.

0.01 part per million in meat, fat, and meat byproducts of poultry.

§ 180.178 Ethoxyquin; tolerances for residues.

A tolerance of 3 parts per million is established for residues of the plant regulator ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) from preharvest or postharvest use in or on apples and pears.

(See also 21 CFR 172.140, 573.380, and 573.400.)

§ 180.179 Tartar emetic; tolerances for residues.[1]

A tolerance of 3.5 parts per million is established for residues of the insecticide tartar emetic, calculated as combined antimony trioxide, in or on each of the following raw agricultural commodities: Citrus fruit, grapes, onions.

§ 180.180 Orthoarsenic acid.

A tolerance of 4 parts per million of combined As_2O_3 is established for residues of the defoliant orthoarsenic acid in or on the raw agricultural commodity cottonseed.

§ 180.181 CIPC; tolerances for residues.

Tolerances are established for residues of the plant regulator and herbicide CIPC (isopropyl m-chlorocarbanilate) and its metabolite 1-hydroxy-2-propyl 3'-chlorocarbanilate (calculated as CIPC) in or on the following raw agricultural commodities:

[1] See footnote 2 to § 180.148.

Commodity	Parts per million
Potatoes (POST H)	50
Soybeans	0.2

[43 FR 52487, Nov. 13, 1978]

§ 180.182 Endosulfan; tolerances for residues.

Tolerances are established for the total residues of the insecticide endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) and its metabolite endosulfan sulfate (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide) in or on the raw agricultural commodities:

Commodities	Parts per million
Alfalfa, fresh	0.2
Alfalfa, hay	1.0
Almonds	0.2(N)
Almonds, hulls	1.0
Apples	2.0
Apricots	2.0
Artichokes	0.1
Barley, grain	0.1(N)
Barley, straw	0.2(N)
Beans	2.0
Beets, sugar, without tops	0.1(N)
Blueberries	0.1(N)
Broccoli	2.0
Brussels sprouts	2.0
Cabbage	2.0
Carrots	0.2
Cattle, fat	0.2
Cattle, mbyp	0.2
Cattle, meat	0.2
Cauliflower	2.0
Celery	2.0
Cherries	2.0
Collards	2.0
Corn, sweet (K + CWHR)	0.1
Cottonseed	1.0
Cucumbers	2.0
Eggplant	2.0
Figs	0.2(N)
Goats, fat	0.2
Goats, mbyp	0.2
Goats, meat	0.2
Grapes	2.0
Hogs, fat	0.2
Hogs, mbyp	0.2
Hogs, meat	0.2
Horses, fat	0.2
Horses, mbyp	0.2
Horses, meat	0.2
Kale	2.0
Lettuce	2.0
Macadamia nuts	0.2(N)
Melons	2.0
Milk fat (1.3% whole milk)	0.5
Mustard greens	2.0
Mustard seed	0.2(N)
Nectarines	2.0
Oats, grain	0.1(N)
Oats, straw	0.2(N)
Peaches	2.0
Peas	2.0
Peas, succulent	1.0
Pecans	0.2(N)
Peppers	2.0
Pineapples	2.0
Plums	2.0
Potatoes	0.2(N)
Prunes	2.0
Pumpkins	2.0
Rape seed	0.2(N)
Raspberries	0.1
Rye, grain	0.1(N)
Rye, straw	0.2(N)
Safflower seed	0.2(N)
Sheep, fat	0.2
Sheep, mbyp	0.2
Sheep, meat	0.2
Spinach	2.0
Squash, summer	2.0
Squash, winter	2.0
Strawberries	2.0
Sugarcane	0.5
Sunflower seed	2.0
Sweet potatoes	0.2
Tomatoes	2.0
Turnips, greens	2.0
Walnuts	0.2(N)
Watercress	2.0
Wheat, grain	0.1(N)
Wheat, straw	0.2(N)

[46 FR 32988, July 20, 1981]

§ 180.183 0,0-Diethyl S-[2-(ethylthio)ethyl] phosphorodithioate; tolerances for residues.

Tolerances are established for the combined residues of the insecticide *0,0*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate and its cholinesterase-inhibiting metabolites, calculated as demeton, in or on the following raw agricultural commodities:

Commodities	Parts per million
Alfalfa, fresh	0.5
Alfalfa, hay	12.0
Asparagus	0.1
Barley, fodder, grain	5.0
Barley, grain	0.75
Barley, straw	5.0
Beans, dry	0.75

E. TOXICOLOGICAL ISSUES

E. TOXICOLOGICAL ISSUES

In common with the other ethylene bisdithiocarbamate fungicides (the EBDC's maneb, zineb, metiram, nabam and Amobam), preparations of mancozeb contain irreducible amounts of an acknowledged goitrogen, teratogen and oncogen, namely, ethylenethiourea (ETU), both as a contaminant in manufacturing and formulation, as well as an inevitable degradation product/metabolic derivative in their usage. The major toxicological concern of exposure to mancozeb (and to the other EBDC's) has reflected the hazard to the human thyroid of this conversion (as enunciated in the Decision Document concluding the EBDC RPAR, dated October 14, 1982, which based the risk assessment of these pesticides on the ETU component). Organ systems other than the thyroid, however, may also be targets for the biological activity of these pesticides. As dicussed above, for example (SECTION A: TOXICOLOGICAL PROFILE), systemic effects have been reported in subchronic feeding/inhalation studies at dose levels one-half to one-fifth those affecting thyroid functions/tissue cells (e.g., in rat kidneys, by the presence of pigment granules, accompanied by renal tubular degeneration; in the dog genital system by prostatic hypogenesis). Adequate long-term (chronic) studies are not available to assess either the validity (or consequences) of these nonthyroid effects, nor whether they reflect a singular mechanism of action, or several diverse mechanisms, nor indeed whether they are referable to the parent EBDC molecule per se (and/or other intermediary metabolites, such as ethylene bisisothiocyanate-sulfide, EBIS) or secondary to conversion to ETU (and/or its metabolites).

These (short-term) studies, however, are neither sufficient nor adequate to satisfy concerns for potential long-term toxicological effects of mancozeb (as well as the other EBDC's), including its oncogenicity potential (as discussed both in the EBDC RPAR Decision Document cited above, and more recently in the August 8, 1986 summary of the weight-of-the-evidence and oncogenic properties of ETU: Memorandum, Hauswirth to the Toxicology Branch Peer Review Committee). Evidence for the chronic toxicity and oncogenoncogenicity of ETU (and other EBDC's) has also recently been summarized (the Hauswirth memorandum, op. cit.). While at the present time a definitive quantitative assessment is not possible, nor those quantitative interrelationships linking ETU risks to EBDC potential, a qualitative characterization of ETU hazard can be made (see tabulation of mancozeb/ETU effects parameters, ff). From these assessments, ETU is demonstrably an oncogen in rodents, as well as inducing dsyfunction and nononcogenic lesions in a variety of organ systems (apparently species-specific), the most sensitive of which is probably the rat thyroid (but awaiting NTP's ETU bioassay results in both rats and mice). Mutagenicity testing results of ETU are equivocal for an initiating event

(summarized in a document accompanying the Hauswirth memorandum, as well as previously by Mauer and others in previous reviews), except in the presence of nitrites such as $NaNO_2$ (generating N-nitroso-ETU), whereas other evidence suggests a promoter (epigenetic) activity (although this evidence is not strong). Finally, mancozeb has not been assayed for oncogenicity (representing a data gap for this EBDC), and its mutagenicity profile (discussed above) is also essentially negative for direct interaction with genetic material/mechanisms (adequate promotional assays are not available).

From the foregoing, at least two major toxicological issues must be resolved:

1. Is ETU the sole active substance accounting for all the chronic effects of mancozeb treatment? If so, what is its mechanism of action?

2. If not, is the parent molecule (and/or other non-ETU derivatives) also responsible for some of the (species-specific) activity? If so, what is its (their) mechanism(s) of action?

Finally, the concern for the potential teratogenicity of mancozeb must be satisfied by an adequate study in a second species. Although ETU has demonstrated developmentally toxic/teratogenic effects in rats and hamsters (producing a wide variety of abnormalities in the central nervous, skeletal, and urogenital systems at dosages that are not maternally nor fetally toxic), evidence to date indicates that mancozeb is not a primary developmental toxicant or teratogen, at least in the single adequate oral study in rats (A/D ratio = 0.25). The A/D ratio in an older, inadequate, study in rabbits was = 0.1; the registrant will be submitting a new rabbit study to meet current Guidelines). Hence, this is another area in which caution is required in equating health hazard risks from the "parent" EBDC molecule to one (but perhaps not the only) active moiety of mancozeb.

NB:

To date (at the time of preparation of this Standard), the lowest NOEL's for nononcogenic mancozeb effects have been recorded in the dog, namely, (1) 100 ppm (= calculated actual intake of 3 mg/kg/day) for nonthyroid systemic effects in a recently submitted adequate subchronic study (in which the next highest dose, 1000 ppm, decreased food consumption and body weight, and caused cortical lymphoid depletion in the thymus as well as prostatic hypogenesis); (2) 100 ppm (= inferred intake of 2.5 mg/kg/day) for thyroid dysfunction (decreased I^{131} uptake and thyroid hyperplasia) in an older supplementary study, which awaits confirmation in a repeat study.

MCZB/ETA EFFECTS PARAMETERS* (ppm)

ORGAN SYSTEM	TOX. EFFECT	RAT				MOUSE			
		MCZB		ETU		MCZB		ETU	
		NOEL	LEL	NOEL	LEL	NOEL	LEL	NOEL	LEL
THYROID	FUNCTION	(100-) 125	250	(<) 5	5	100	1000	10	100
	ONCO	N/A	N/A	125	175	NEGATIVE		N/A	
LIVER	FUNCTION	N/A	N/A	NEGATIVE		1000	10,000	10	100
	ONCO	N/A	N/A	NEGATIVE		N/A	N/A	< 646	646
KIDNEY	FUNCTION	60	125	N/A	N/A	N/A	N/A	N/A	N/A
	ONCO	N/A				N/A			

*In absence of adequate chronic (long-term) studies.
N/A=not available.

F. DATA REQUIREMENTS

SUMMARY TABLES

(A / B)

TABLE A
GENERIC DATA REQUIREMENTS FOR MANEB

Data Requirement	Composition 1/	Use Patterns 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
§158.135 Toxicology					
ACUTE TESTING:					
81-1 - Acute Oral - Rat	TGAI		YES	047146 093926 142522	NO
81-2 - Acute Dermal	TGAI		YES	047146 093927 142522	NO
81-3 - Acute Inhalation - Rat	TGAI		YES	047146 093923 145996	NO
81-4 - Eye Irritaion - Rabbit	TGAI		YES	142522	NO
81-5 - Dermal Irritation - Rabbit	TGAI		YES	047146 093927 142522	NO
81-6 - Dermal Sensitization - Guinea Pig	TGAI		NO		YES
81-7 - Acute Delayed Neurotoxicity - Hen 4/	TGAI		—		—
SUBCHRONIC TESTING:					
82-1 - 90-Day Feeding -					
Rodent	TGAI		YES	IMM203	NO
Non-rodent	TGAI		YES	IMM204	NO

TABLE A
GENERIC DATA REQUIREMENTS FOR MANCOZEB

Data Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
§158.135 Toxicology (Cont.)					
82-2 - 21-Day Dermal-	TGAI		NO		YES
82-3 - 90-Day Dermal-	TGAI		NO		YES
82-4 - 90-Day Inhalation -	TGAI		YES	1MM205	NO
82-5 - 90-Day Neurotoxicity-	TGAI				
CHRONIC TESTING:					
83-1 - Chronic Toxicity -					
Rodent	TGAI		NO		YES
Non-rodent	TGAI		NO		YES
83-2 - Oncogenicity Study -			~~YES~~		~~YES~~
Rat	TGAI		NO		YES
Mouse	TGAI		NO		YES
83-3 - Teratogenicity -					
Rat	TGAI		YES	093929	NO
Rabbit	TGAI		NO		YES
83-4 - Reproduction -	TGAI		NO		YES

TABLE A
GENERIC DATA REQUIREMENTS FOR MANCOZEB

Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
1.135 Toxicology (continued)					
MUTAGENICITY TESTING					
84-2 - Gene Mutation	TGAI		YES	IMM208	NO
84-2 - Chromosomal Aberration	TGAI		YES	IMM208	NO
84-2 - Other Mechanisms of Mutagenicity	TGAI		PARTIALLY 5/	IMM208	YES 5/
SPECIAL TESTING					
84-1 - General Metabolism	PAI or PAIRA		YES	IMM209	NO
84-2 - Domestic Animal Safety	Choice		NO		YES
85-3 - DERMAL ABSORPTION RAT			NO		YES

Composition: TGAI Technical Grade Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient, RAdiolabelled; Choice = Choice of several test substances determined on a case-by-case basis.
The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Non-Food; C = Aquatic, Food Crop; D = Aquatic, Non-Food; E = Greenhouse, Food Crop; F = Greenhouse, Non-Food; G = Forestry; H = Domestic Outdoor; I = Indoor; IP = Industrial Preservative.
3/ Unless otherwise specified data must be submitted no later than six months after publication of this Standard

4/ Not required (mancozeb is not an organophosphate)
5/ The UDS assay in primary rat hepatocytes must be repeated. Additionally, gene-promotion assays in vitro must be submitted.

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TABLE B

PRODUCT SPECIFIC DATA REQUIREMENTS FOR MANUFACTURING-USE PRODUCTS CONTAINING MANCOZEB

ita Requirement	Composition 1/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 2/
158.135 Toxicology				
ACUTE TESTING				
81-1 - Acute Oral - Rat	MP	YES	097182 126511 1MM201	NO
81-2 - Acute Dermal	MP	YES	097182 126511 1MM201	NO
81-3 - Acute Inhalation - Rat	MP	YES	1MM202	NO
81-4 - Primary Eye Irritation - Rabbit	MP	YES	097182 126511 1MM201	NO
81-5 - Primary Dermal Irritation - Rabbit	MP	YES	097182 126511 1MM201	NO
81-6 - Dermal Sensitization Guinea pig	MP	NO		YES

1/ Composition: MP = Manufacturing-use product.

2/ Unless otherwise specified data must be submitted no later than six months after publication of this Standard

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Byers, M.J.; Scribner, H.E. Dithane M-45, in vitro unscheduled DNA synthesis. (Unpublished study No. 84R-280 prepared and submitted by Rohm & Haas Co., Spring House, PA; dated May 29, 1985.) EPA Accession No. 258044.

Sames, J.L.; McLeod, P.L.; Doolittle, D.J. Dithane M-45 in vivo cytogenetic study in rats. (Unpublished study No. 84R-246 prepared and submitted by Rohm & Haas, Spring House, PA; dated December 21, 1984.) EPA Accession No. 259044.

Ivett, J.L.; Myhr, B.C. Mutagenicity evaluation of Dithane M-45 Fungicide lot No. 0842 in an in vitro sister chromatid exchange assay in Chinese hamster ovary (CHO) cells. (Unpublished study No. 84RC-60 prepared by Litton Bionetics, Inc., Kensington, MD, for Rohm & Haas, Spring House, PA; dated March 1985.) EPA Accession No. 259044.

IHMZ08 (cont'd) McGlynn-Kreft, A.M.; McCarthy, K.L. Dithane M-45 mammalian cell transformation test. (Unpublished study No. 84R-055 prepared and submitted by Rohm & Haas Co., Spring House, PA; dated November 19, 1984.) EPA Accession No. 259044.

McLeod, P.L.; Doolittle, D.J. Dithane M-45 mammalian cell transformation test for promotion. (Unpublished study No. 84R-297 prepared and submitted by Rohm & Haas Co., Spring House, PA; dated May 29, 1985.) EPA Accession No. 259044.

IHMZ09 DiDonato, L.J.; Longacre, S.L. Mancozeb pharmacokinetic study in rat (unpublished study No. 85R-123); Prepared by Rohm & Haas Company, Philadelphia, PA; dated May 21/22, 1985. Accession Nos. 262834 and 262835.

Longacre, S.L. Summary of ETU and EBDC analyses in plasma, liver, and thyroid after mancozeb administration (unpublished study No. 85R-123); Prepared by Rohm & Haas Company, Philadelphia, PA; dated May 21/22, 1985. Accession Nos. 262834 and 262835.

Nelson, S.S. Metabolism of C-14 mancozeb in rat (unpublished study No. 31H-86-02). Prepared by Rohm & Haas Company, Philadelphia, PA; dated May 21/22, 1985. Accession Nos. 262834 and 262835.

H. ONE-LINERS

Tox Chem No. 913A (mancozeb) File Last Updated 10/21/85 Current Date 09/15/86

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
REGISTRATION STANDARD September, 1986					005425
Teratology - rat Booz,Allen & Hamilton/ Snell. Project # 10065-0009, 5/29/80 (MRID 093929)	Technical (83% ai)	246663	Doses tested: 0, 2, 8, 32, 128, 512 mg/kg/day Teratogenic NOEL = 128 mg/kg Teratogenic LEL = 512 mg/kg (incomplete ossification of skull and ribs; spinal cord hemorrhage; dilated ventricles) Fetotoxic NOEL = 128 mg/kg Fetotoxic LEL= 512 mg/kg (increased resorptions) Maternal NOEL = 32 mg/kg Maternal LEL = 128 mg/kg (decreased body weight) A/D ratio = 0.25		Minimum 002169 Minimum 005425
Teratology - rabbit Brown Biological Labs; Rohm & Haas Study #68-RC-1013, August 8, 1968. (MRID IMMZ07)	Technical (80% ai)		Doses tested: 0, 25, 250 mg/kg/day. Teratogenic NOEL > 250 mg/kg Fetotoxic NOEL > 250 mg/kg Maternal NOEL = 25 mg/kg Maternal LEL = 250 mg/kg (decreased body weight) A/D ratio = 0.1.		Supplementary 005425
3-Generation reproduction - rat Medical College of Virginia, 1965. (MRID 080715)	Technical (86% ai)		Levels tested: 0, 25, 100, 1000 ppm. Parental toxicity NOEL > 1000 ppm Reproductive NOEL = 100 ppm (5 mg/kg/day) Reproductive LEL = 1000 ppm (decreased fertility) Fetal NOEL > 1000 ppm. No individual animal data.		002493 Supplementary 005425
Functional/morphological thyroid study (Addendum to Reproductive/ study). Medical College of Virginia, 1965. (MRID 080715/080713)	Technical (86% ai)		Levels tested: 0, 100, 300, 1000 ppm (to the F1b/F2a) NOEL = 300 ppm LEL = 1000 ppm (50 mg/kg/day) (increased thyroid/body weight; hyperplasia Incomplete report[illegible] and [illegible]		002493 Supplementary 005425

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Tox Chem No. _____ File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Subchronic (90-day) feeding - rat. Rohm & Haas, Study #86R-167, February 27, 1986. (MRID 1MMZ03)	Technical (84% ai)	261536	Levels tested: 0, 30, 60, 125, 1000 ppm in S-D rats. Systemic NOEL = 60 ppm (3.5/4.4 mg/kg/day, respectively) Systemic LEL = 125 ppm (7.4/9.2 mg/kg/day); renal tubular degeneration in males. Thyroid NOEL = 125 ppm Thyroid LEL = 250 ppm (decreased T4/TSH levels in females)		Minimum 005318
Subchronic (90-day) feeding - dog. Hazleton , Study # 86RC-7, February 26, 1986. (MRID 1MMZ04)	Technical (83.3% ai)	261537	Levels tested: 0, 10, 100, 1000, 5000 ppm in Beagles. Systemic NOEL = 100 ppm (3 mg/kg/day) Systemic LEL = 1000 ppm (29 mg/kg/day (decreased food consumption/ body weight gains; cortical lymphoid depletion in thymus; prostatic hypogenesis.) Thyroid NOEL = 1000 ppm Thyroid LEL = 5000 ppm (102/109 mg/kg/day in males/females, resp) (thyroid follicular cell hyperplasia, decreased T3/T4; hypercholesterolemia/ hyperbilirubinemia; decreased food consumption/body weight)		Minimum 005318
Subchronic (90-day) inhalation - rat. Rohm & Haas, Study #86R-003, February 27, 1986. (MRID 1MMZ05)	Technical (83.3 % ai)	261539 (261538)	Concentrations: 0, 20, 80, 320 mg/m^3, (nominal (respirable concentrations = 0, 8, 36, 144 mg/m^3) to S-D rats. NOEL = 8 mg/m^3 (respirable) LEL = 36 mg/m^3 (respirable) (granular pigment in renal tubules, both sexes) Thyroid NOEL = 36 mg/m^3 Thyroid LEL = 144 mg/m^3 (respirable) (decreased T4 in females, accompanied by follicular epithelial hyperplasia.		Minimum 005318

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Tox Chem No. _____ File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Subchronic (8-week) feeding/neurotoxicity - chicken Central Insitute for Nutrition/Food Res., The Netherlands, Report Nr. R-3290, October, 1970. (MRID 129288)	DITHANE M-45		Levels tested: 0, 0.02%, 0.10%, 0.20% of the diet to male/female "broiler" chickens (= 0, 200, 1000, 2000 ppm) Systemic NOEL = 0.02% (200 ppm) Systmeic LEL= 0.10% (1000 ppm) (leg paralysis, decreased BW gain in males, increased relative thyroid weight in both sexes, increased relative liver weight in males; goiter with colloid enlargement in both sexes) No individual animal data; no reporting on neural histology.		Supplementary 005425
Subchronic (90-day) feeding - mice. Rohm & Haas, Study #80R-124, February 18, 1985. (MRID 1MMZ10)	Technical (83.1 % ai)	259888	Levels tested: 0, 10 , 100, 1000 and 10,000 ppm in CD-1 mice NOEL = 100 ppm LEL = 1000 ppm (increased incidence of thyroid follicular cell hyperplasia/hypertrophy; decreased hepatic aminopyrine N-demethylase).		005038 Supplementary 005425
Chronic feeding - rat. Medical College of Virginia, November 9, 1965. (MRID 080713)	Technical (86% ai)		Levels tested: 0, 25, 50, 100, 1000 ppm. Systemic NOEL = 100 ppm Systemic LEL = 1000 ppm (thyroid hyperplasia in both sexes). Poor survival necessitated termination at 90 weeks (21 months); inadequate histopahthology and clinical chemistries; no individual animal data.		002493 Supplementary 005425
Chronic (two-year) feeding - dog. Medical College of Virginia, 1965. (MRID 080714) (December 1	Technical (86 % ai)		Levels tested: 0, 25, 100, 1000 ppm. NOEL (thryoid) = 100 ppm (2.5 mg/kg/day) LEL = 1000 ppm (25 mg/kg/day); decreased I-131 uptake, thryoid hyperplasia. No neoplasia reported. Summary data only.		Supplementary 005425

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Tox Chem No. _____ File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism - rat. Rohm & Haas, Study #85R-123 (31H-86-02), May 21/22, 1986. (MRID IMMZ09)	C-14 technical (84.4% ai)	262834/ 262835	Doses tested: 1.5. 100 mg/kg (x1, oral) 50% of oral dose absorbed; excreted equally in urine/feces; rapidly metabolized and/or degraded to ETU (major component) and intermediates (ETD, EBIS, EDA, etc.); accumulates in major organs, highest in thyroid. Residue analysis for ETU = 1 ppm in thyroid during 24 hr. after high dose (only); ~~in~~ undetectable there after.		Minimum 005425
Mutagenicity: gene mutation in bacteria (Ames Assay). Rohm & Haas, Reports #'s 84R0059. 84R0060, June 21, 1984. (MRID IMMZ08)	Technical (88 % ai)	25[illegible] ~~25[illegible]~~	Negative for reversion in Ames S. typhimurium strains up to cytotoxic concentrations, with/without activation. (250 ug/plate),		Acceptable
Mutagenicity; gene mutation in host-mediated assay (mice). Rohm & Haas, Report #84RC-48, M July 1, 1984. (MRID IMMZ08)	Technical (88%ai)	25[illegible]044	Negative for reversion of S. typhimurium G46 incubated in mice treated up to 5000 mg/kg.		Acceptable
Mutagenicity: gene mutation in mammalian cells in vitro (CHO/HGPRT). Rohm & Haas, Report #84R-207, February 11, 19 1985. (MRID IMMZ08)	Technical (88% ai)	25[illegible]044	Negative for foreward mutation up to cytotoxic concentrations (450 ug/ml), with/without activation.		Acceptable
Mutagenicity: chromosome damage in vitro (CHO/SCE). Rohm & Haas, Report #84RC-60, March, 1985. (MRID IMMZ08)	Technical (88%ai)	25[illegible]044	POSITIVE for inducing sister-chromatid exchanges at non-toxic doses in both non-activated and activated assays (7.5 ug/ml ~~and higher~~) (10 ug/ml)		Acceptable

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Tox Chem No. _____ File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenicity: Chromosome damage in vivo (rat BM) Rohm & [illegible]s, Report # 8[illegible]46, December 21, 1[illegible] (MRID IMMZ08)	Technical (88% ai)	~~259044~~ 259044	Negative for chromosome aberrations (clastogenesis) in bone marrow cells up to toxic doses (4.4 g/kg)		Acceptable
Mutagenicity: DNA damage/repair in vitro (HPC/UDS) Rohm & Haas Report #84R-280, December 21, 1984 (MRID IMMZ08)	Technical (88% ai)	259044	Presumptively positive for unscheduled DNA synthesis in rat hepatocyte cultures treated at 1, 2.5 and 5.0 ug/ml. Procedural problems indicate assay should be repeated.		Inconclusive
Mutagenicity: other mechanisms (transformation in vitro) Rohm & Haas, Report #85R-055, November 19, 1984 (MRID IMMZ08)	Technical (88% ai)	259044	Negative at concentrations up to cytotoxicity (0.5 ug/ml) for transforming C3H $10T_{\frac{1}{2}}$ cells in vitro		Acceptable
Mutagenicity: other mechanisms (promotion in vitro) Rohm & Haas, Report #84R-297, March 29, 1985. (MRID IMMZ08)	Technical (88% ai)	259044	Reported negative for carcinogen-initiated cells exposed ~~(control, the known promoter, TPA). However, tested at only one dose (non-toxic), which may be insufficient~~	non-toxic only one dose Unacceptable	

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD50 - rat. Rohm & Haas, Report # 83R-213B, September 24, 1984. (MRID IMMZ02)	Technical (80% ai)	259044	LD50 (males) > 5000 mg/kg No females were tested.	IV	Supplementary 005425
Acute oral LD50 - rat. Rohm & Haas, Report #83R-218, September 21, 1984. (MRID IMMZ02)	Technical (80% ai)	259044	LD50 (males) > 5000 mg/kg No females were tested	IV	Supplementary 005425
Acute oral LD50 - mice. Rohm & Haas, Report #213A, September 24, 1984. (MRID IMMZ01)	Technical (80% ai)	259044	LD50 (males) > 5000 mg/kg No females were tested.	IV	Supplementary 005425
Acute oral LD50 - rat. Rohm & Haas, Report #83R-086A, June 20, 1983. (MRID 142522)	Technical (72.6% ai)	xx	LD50 (males/females) > 5000 mg/kg	IV	Minimum 005425
Acute oral LD50 - rat. University of Miami, (Received January 11, 1965) (MRID 047146)	"Zimaneb" technical (80% ai)		LD50 (males/females) = 4500 mg/kg (3600-5700 mg/kg)	III	Minimum 005425
Acute dermal LD50 -rabbit Rohm & Haas, Report #83R-086A, June 20, 1983. (MRID 142522)	Technical (72.6% ai)		LD50 (males/females) > 5000 mg/kg	III	Minimum 005425
Acute/subacute dermal - rabbit. University of Miami (Received January 11, 1965). (MRID 047146)	"Zimaneb" techical (80% ai)		LD50 (males/females) > 10,000 mg/kg. No effect of five-days' treatment at 5000 mg/kg/day.	III	Minimum 005425

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute inhalation LC50 - rat. University of Miami (Received January 11, 1965) (MRID 047146)	"Zimaneb" Technical (80% ai)		LC50 (males/females) > 6.85 mg/L (ODT) Acthal chamber concentration and respirable particle size not determined.	IV	Supplementary 005425
Acute inhalation LC_{50} - rat; Rohm & Haas Labs.; #79R-132; 12/18/80	a coordination product of zinc ion & manganese ethylene bis-dithiocarbamate 35% (707-156)	244505	LC_{50} > 0.35 mg/L - only dose tested (gravimetric concentration)	II	~~Minimum~~ 001243
Acute inhalation LC_{50} - rat; Report #81R-171; 1/21/80	Manganese 16% Zinc 2% Ethylene bis-dithiocarbamate ion 62%	246662	LC_{50} greater than 5.14 mg/L.	IV	Guideline 002803

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Tox Chem No. _____ File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD_{50} - rat; Haskell Lab.; #876-80; October 22, 1980	A coordination prod. of zinc ion and manganese ethylene-bis dithiocarbamate 35%	244298	LD_{50} > 5000 mg/kg (only dose tested)	IV	Guideline 000809
Acute oral LD_{50} - rat; Rohm and Haas; Report #79R-180; 1/21/80	Manganese 16% Zinc 2% Ethylene bis-dithiocarbamatetion 62%	246662	LD_{50} greater than 5 g/kg (M)	IV	Minimum 002803
Acute oral LD_{50} - rat; Rohm and Haas Co.	Mancozeb Flowable (36% a.i.)	238564	LD_{50} > 5 gm/kg (male)	IV	Minimum 003245
Acute oral LD_{50} - rat; Rohm & Haas Labs; 3/4/68	Dithane M-45 Technical 86% a.i.		LD_{50} > 8000 mg/kg Levels tested = 4, 6, 8, g/kg	IV	002493
Acute oral LD_{50} - rat; Rohm & Haas Co; 6/5/79	35% a.i.	238564	LD_{50} > 5000 mg/kg (only dose tested)	IV	Minimum 002494
Acute dermal LD_{50} - rabbit; Haskell Lab.; #875-80; 10/14/80	A coordination prod. of zinc ion and manganese ethylene-bis dithiocarbamate 35%	244298	LD_{50} > 2000 mg/kg (only dose tested)	III	Guideline 000809
Acute dermal LD_{50} - rabbit; Rohm & Haas; Report #79R-180; 1/21/80	Manganese 16% Zinc 2% Ethylene bis-dithiocarbamate ion 62%	246662	LD_{50} greater than 5 g/kg (M)	III	Minimum 002803
Acute dermal LD_{50} - rabbit; Rohm & Haas Co.	Mancozeb Flowable (36% a.i.)	238564	LD_{50} > 5 gm/kg (male) (only dose tested) (8)	III	Minimum 003245
Acute dermal LD_{50} - rabbit; Rohm & Haas Co.	35% a.i.	238564	LD_{50} > 5000 mg/kg (only dose tested)	III	Minimum

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation - rabbit,. Rohm & Haas, Study #83R-086A, June 20, 1983 (MRID 142522)	Technical (72.6% ai)		PIS (72 hr) = 2.3	III	Minimum 005425
Primary eye irritation - rabbit; Haskell Lab.; #826-80; 8/25/80	A coordination prod. of zinc ion and manganese ethylene-bis - dithiocarbamate 35%	244298	Corneal opacity in 6/6 treated, unwashed eyes and 3/3 treated washed eyes. Conjunctive redness, swelling, and discharge in most animals.	II	Guideline 000809
Primary eye irritation - rabbit; Rohm & Haas Co.	Mancozeb Flowable (36% a.i.)	238564	PIS = 0	IV	Guideline 003245
Primary eye irritation - rabbit; Rohm & Haas; 6/5/79	35% a.i.	238564	PIS = 0 at 24 hours. No corneal opacity	III	Guideline 002494
Primary dermal irritation - rabbit; Haskell Lab.; #825-80; 8/25/80	A coordination prod. of zinc ion and manganese ethylene-bis dithiocarbamate 35%	244298	Slight to well defined erythema and edema at 24 hours and persisted in 3/6 animals thru day 9, but all irritation clear by day 10.	III	Guideline 000809
Primary dermal irritation - rabbit; Report #79R-180; 1/21/80	Manganese 16% Zinc 2% Ethylene bis-dithiocarbamate ion 62%	246662	At 24 hrs. slight erythema. At 72 hrs. slight erythema. PIS = 0.5	IV	Guideline 002803
Primary dermal irritation - rabbit; Rohm and Haas Co.	Mancozeb Flowable (36% a.i.)	238564	PIS = 0.4 /8.0	IV	Guideline 003245
Primary dermal irritation - rabbit; Rohm & Haas; 6/5/79	35% a.i.	238564	PIS = 0.4	IV	Guideline 002494

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Chem. No. _____ File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary skin irritation rabbit. Rohm & Haas, Study #83R-086A, June 20, 1983 (MRID 142522)	Technical (72.6% ai)		PIS (72 hr) = 0.2	IV	Minimum 005425
Primary skin irritation-rabbit University of Miami (Received January 11, 1965) (MRID 047146)	"Zimaneb" technical (80% ai)		No irritation reported from treatment at 2000 mg/kg. Too few animals tested.		Supplementary 005425
Dermal absorption - rat. Rohm & Haas (Springhouse Research Labs., Study #34F-80-9, May 8, 1980. MRID 127950)	Dithane M-45 (8.3% ai)	250063	Approximately 1% mancozeb in a 10 mg dose of Dithane M-45 commercial formulation (8.3%) is absorbed through the skin of female rats following a 6-hour application.		Acceptable 003997 Supplementary 005425

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Report of the Analysis of Dithane M-45 and ETU [in]...Urine Samples from Applicators and Mixer-Loaders... in Ohio. Dept. of Entomology Penn. State Univ. Feb. 17, 1983 (MRID 130638)	Dithane M-45 (mancozeb)	none	In two trials, ETU, but not the parent mancozeb, was detected in the urine of aerial applicators (pilots) at a level of 0.2ppm. These two trials were conducted in Michigan and Minnesota; the urine of mixer-loaders and home gardners was negative. All trials in other states were also negative. These results suggest that agricultural use of mancozeb, and probably other EBDC's, results in at least some applicator exposure to the parent compound and the common metabolite, ETU. Quantification of this exposure is not possible from these data, as presented.	N/A	004726 005425
Human exposure (MRID 130638)	Dithane M-45		No adverse effects observed in 54 men exposed during the manufacturing of Dithane M-45.		002493 003244 005425

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File Last Updated 10/21/85 Current Date 09/15/86

Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
				005425
Technical (83% ai)	246663	Doses tested: 0, 2, 8, 32, 128, 512 mg/kg/day Teratogenic NOEL = 128 mg/kg Teratogenic LEL = 512 mg/kg (incomplete ossification of skull and ribs; spinal cord hemorrhage dilated ventricles) Fetotoxic NOEL = 128 mg/kg Fetotoxic LEL= 512 mg/kg (increased resorptions) Maternal NOEL = 32 mg/kg Maternal LEL = 128 mg/kg (decreased body weight) A/D ratio = 0.25		Minimum 002169 Minimum 005425
echnical 30% ai)		Doses tested: 0, 25, 250 mg/kg/day. Teratogenic NOEL > 250 mg/kg Fetotoxic NOEL > 250 mg/kg Maternal NOEL = 25 mg/kg Maternal LEL = 250 mg/kg (decreased body weight) A/D ratio = 0.1.		Supplementary 005425
chnical 6% ai)		Levels tested: 0, 25, 100, 1000 ppm. Parental toxicity NOEL > 1000 ppm Reproductive NOEL = 100 ppm (5 mg/kg/day) Reproductive LEL = 1000 ppm (decreased fertility) Fetal NOEL > 1000 ppm. No individual animal data.		002493 Supplementary 005425
hnical 6% ai)		Levels tested: 0, 100, 300, 1000 ppm (to the F1b/F2a) NOEL = 300 ppm LEL = 1000 ppm (50 mg/kg/day) (increased thyroid/body weight; hyperplasia Incomplete reporting and		002493 Supplementary 005425

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File Last Updated _____ Current Date _____

te	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
85R-27,	Technical (84% ai)	261536	Levels tested: 0, 30, 60, 125, 1000 ppm in S-D rats. Systemic NOEL = 60 ppm (=3.5/4.4 mg/kg/day, respectively) Systemic LEL = 125 ppm (=7.4/9.2 mg/kg/day; renal tubular degeneration in males. Thyroid NOEL = 1000 ppm Thyroid LEL = 1000 ppm (decreased T4/T3 levels in females)		Minimum 005316
g. ry	Technical (83.3% ai)	261537	Levels tested: 0, 10, 100, 1000, 5000 ppm in Beagles. Systemic NOEL = 100 ppm (3 mg/kg/day) Systemic LEL = 1000 ppm (29 mg/kg/day (decreased food consumption/ body weight gain; cortical lymphoid depletion in thymus; prostatic hypogenesis. Thyroid NOEL = 1000 ppm Thyroid LEL = 5000 ppm (172/10- day in males/females, (thyroid follicular cell hyperplasia, decreased T3/T4; hypercholesterolemia/ hyperbilirubinemia; decreased food consumption/body weight)	BEST AVAILABLE COPY	Minimum 005316
t. 86R-	Technical (83.3 % ai)	261539 (261538)	Concentrations: 0, 20, 80, 320 mg/m^3, (nominal (respirable concentrations = 0, 8, 36, 144 mg/m^3) to S-D rats. NOEL = 8 mg/m^3 (respirable) LEL = 36 mg/m^3 (respirable) (granular pigment in renal tubules, both sexes) Thyroid NOEL = 36 mg/m^3 Thyroid LEL = 144 mg/m^3 (respirable) (decreased T4 in females, accompanied by follicular epithelial hyperplasia.		Minimum 005316

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te	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
kicity	DITHANE M-45		Levels tested: 0, 0.02%, 0.10%, 0.20% of the diet to male/female "broiler" chickens (= 0, 200, 1000, 2000 ppm) Systemic NOEL = 0.02% (200 ppm) Systmeic LEL= 0.10% (1000 ppm) (leg paralysis, decreased BW gain in males, increased relative thyroid weight in both sexes, increased relative liver weight in males; goiter with colloid enlargement in both sexes) No individual animal data; no reporting on neural histology.		Supplementary 005425
80R- 8,	Technical (83.1 % ai)	259888	Levels tested: 0, 10 , 100, 1000 and 10,000 ppm in CD-1 mice NOEL = 100 ppm LEL = 1000 ppm (increased incidence of thyroid follicular cell hyperplasia/hypertrophy; decreased hepatic aminopyrine N-demethylase).	BEST AVAILABLE COPY	005038 Supplementary 005425
t. ir- r	Technical (86% ai)		Levels tested: 0, 25, 50, 100, 1000 ppm. Systemic NOEL = 100 ppm Systemic LEL = 1000 ppm (thyroid hyperplasia in both sexes). Poor survival necessitated termination at 90 weeks (21 months); inadequate histopahthology and clinical chemistries; no individual animal data.		002493 Supplementary 005425
g. ir- er 1	Technical (86 % ai)		Levels tested: 0, 25, 100, 1000 ppm. NOEL (thyroid) = 100 ppm (2.5 mg/kg/day) LEL = 1000 ppm (25 mg/kg/day); decreased I-131-uptake, thyroid hyperplasia. Neoplasia reported. Summary data only.		Supplementary 005425

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e	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
85R-2), 5.	C-14 technical (84.4% ai)	262834/ 262835	Doses tested: 1.5. 100 mg/kg (x1, oral) 50% of oral dose absorbed; excreted equally in urine/feces; rapidly metabolized and/or degraded to ETU (major component) and intermediates (ETD, EBIS, EDA, etc.); accumulates in major organs, highest in thyroid. Residue analysis for ETU = 1 ppm in thyroid during 24 hr. after high dose (only); ~~in~~ undetectable there after.		Minimum 005425
ta- Ames #'s	Technical (88 % ai)	25[illegible]	Negative for reversion in Ames S. typhimurium strains up to cytotoxic concentrations, with/without activation. (250 ug/plate),		Acceptable
ta- ia- 84FC-84.	Technical (88%ai)	25[illegible]044	Negative for reversion of S. typhimurium G46 incubated in mice treated up to 5000 mg/kg/		Acceptable
ta- n 84F-, 19	Technical (88% ai)	25[illegible]044	Negative for foreward mutation up to cytotoxic concentrations (45 ug/ml), with/without activation .		Acceptable
one 84RC-	Technical (88%ai)	25[illegible]044	POSITIVE for inducing sister-chromatid exchanges at non-toxic doses in both non-activated and activated assays (7.5 ug/ml) ~~and higher~~ (10 ug/ml)		Acceptable

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005425

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te	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
nosome ro # ber 21,	Technical (88% ai)	~~259044~~ 259044	Negative for chromosome aberrations (clastogenesis) in bone marrow cells up to toxic doses (4.4 g/kg)		Acceptable
~~XMR822~~ n vitro 84R- ,	Technical (88% ai)	259044	Presumptively positive for unscheduled DNA synthesis in rat hepatocyte cultures treated at 1, 2.5 and 5.0 ug/ml. Procedural problems indicate assay should be repeated.		Inconclusive
mecha- ma-) #85R- 9,	Technical (88% ai)	259044	Negative at concentrations up to cytotoxicity (0.5 ug/ml) for transforming C3H $10T_{\frac{1}{2}}$ cells in vitro		Acceptable
mecha- #84R- 985.	Technical (88% ai)	259044 259044	Reported negative for carcinogen- ~~xxxxxxxxx~~ initiated cells exposed ~~to~~ only one dose ~~(control, the known promoter, TPA). However, tested at only one dose (non-toxic), which may be insufficient~~	non-toxic only one dose	Unacceptable

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	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
83R-24,	Technical (80% ai)	259044	LD50 (males) > 5000 mg/kg No females were tested.	IV	Supplementary 005425
3R-21,	Technical (80% ai)	259044	LD50 (males) > 5000 mg/kg No females were tested	IV	Supplementary 005425
13A, 4.	Technical (80% ai)	259044	LD50 (males) > 5000 mg/kg No females were tested.	IV	Supplementary 005425
3R-83.	Technical (72.6% ai)	xx	LD50 (males/females) > 5000 mg/kg	IV	Minimum 005425
1,	"Zimaneb" technical (80% ai)		LD50 (males/females) = 4500 mg/kg (3600-5700 mg/kg)	III	Minimum 005425
bit 3R-83.	Technical (72.6% ai)		LD50 (males/females) > 5000 mg/kg	III	Minimum 005425
ec-11,	"Zimaneb" techical (80% ai)		LD50 (males/females) > 10,000 mg/kg. No effect of five-days' treatment at 5000 mg/kg/day.	III	Minimum 005425

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e	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
- Rec 11,	"Zimaneb" Technical (80% ai)		LC50 (males/females) > 6.85 mg/L (ODT) Acttual chamber concentration and respirable particle size not determined	IV	Supplementary 005425
) - s.;	a coordination product of zinc ion & manganese ethylene bis-dithiocarbamate 35% (707-156)	244505	LC_{50} > 0.35 mg/L - only dose tested (gravimetric concentration)	II	~~Minimum~~ 001243
) - :	Manganese 16% Zinc 2% Ethylene bis-dithiocarbamate ion 62%	246662	LC_{50} greater than 5.14 mg/L.	IV	Guideline 002803

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	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
	A coordination prod. of zinc ion and manganese ethylenebis dithiocarbamate 35%	244298	LD_{50} > 5000 mg/kg (only dose tested)	IV	Guideline 000809
	Manganese 16% Zinc 2% Ethylene bisdithiocarbamatetion 62%	246662	LD_{50} greater than 5 g/kg (M)	IV	Minimum 002803
	Mancozeb Flowable (36% a.i.)	238564	LD_{50} > 5 gm/kg (male)	IV	Minimum 003245
68	Dithane M-45 Technical 86% a.i.		LD_{50} > 8000 mg/kg Levels tested = 4, 6, 8, g/kg	IV	002493
	35% a.i.	238564	LD_{50} > 5000 mg/kg (only dose tested)	IV	Minimum 002494
	A coordination prod. of zinc ion and manganese ethylenebis dithiocarbamate 35%	244298	LD_{50} > 2000 mg/kg (only dose tested)	III	Guideline 000809
30	Manganese 16% Zinc 2% Ethylene bisdithiocarbamate ion 62%	246662	LD_{50} greater than 5 g/kg (M)	III	Minimum 002803
	Mancozeb Flowable (36% a.i.)	238564	LD_{50} > 5 gm/kg (male) (only dose tested) (8)	III	Minimum 003245
	35% a.i.	238564	LD_{50} > 5000 mg/kg (only dose tested)	III	Minimum

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Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Technical (72.6% ai)		PIS (72 hr) = 2.3	III	Minimum 005425
A coordination prod. of zinc ion and manganese ethylene-bis - dithiocarbamate 35%	244298	Corneal opacity in 6/6 treated, unwashed eyes and 3/3 treated washed eyes. Conjunctive redness, swelling, and discharge in most animals.	II	Guideline 000809
Mancozeb Flowable (36% a.i.)	238564	PIS = 0	IV	Guideline 003245
35% a.i.	238564	PIS = 0 at 24 hours. No corneal opacity	III	Guideline 002494
A coordination prod. of zinc ion and manganese ethylene-bis dithiocarbamate 35%	244298	Slight to well defined erythema and edema at 24 hours and persisted in 3/6 animals thru day 9, but all irritation clear by day 10.	III	Guideline 000809
Manganese 16% Zinc 2% Ethylene bis-dithiocarbamate ion 62%	246662	At 24 hrs. slight erythema. At 72 hrs. slight erythema. PIS = 0.5	IV	Guideline 002803
Mancozeb Flowable (36% a.i.)	238564	PIS = 0.4 /8.0	IV	Guideline 003245
35% a.i.	238564	PIS = 0.4	IV	Guideline 002494

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Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Technical (72.6% ai)		PIS (72 hr) = 0.2	IV	Minimum 005425
"Zimaneb" technical (80% ai)		No irritation reported from treatment at 2000 mg/kg. Too few animals tested.		Supplementary 005425
Dithane M-45 (8.3% ai)	250063	Approximately 1% mancozeb in a 10 mg dose of Dithane M-45 commercial formulation (8.3%) is absorbed through the skin of female rats following a 6-hour application.		Acceptable 003997 Supplementary 005425

005425

File Last Updated _____ Current Date 10/21/85

Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL.	TOX Category	CORE Grade/ Doc. No.
Dithane M-45 (mancozeb)	none	In two trials, ETU, but not the parent mancozeb, was detected in the urine of aerial applicators (pilots) at a level of 0.2ppm. These two trials were conducted in Michigan and Minnesota; the urine of mixer-loaders and home gardners was negative. All trials in other states were also negative. These results suggest that agricultural use of mancozeb, and probably other EBDC's, results in at least some applicator exposure to the parent compound and the common metabolite, ETU. Quantification of this exposure is not possible from these data, as presented.	N/A	004726 005425
Dithane M-45		No adverse effects observed in 54 men exposed during the manufacturing of Dithane M-45.		002493 003244 005425

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005425

EPA: 68-02-4225
DYNAMAC No. 205-0
October 17, 1986

DATA EVALUATION RECORD

MANCOZEB

Two-Year Chronic Toxicity Study in Dogs

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 10-17-86

005425

EPA: 68-02-4225
DYNAMAC No. 205-0
October 17, 1986

DATA EVALUATION RECORD

MANCOZEB

Two-Year Chronic Toxicity Study in Dogs

REVIEWED BY:

Kumar D. Mainigi, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Kumar D. Mainigi
Date: 10-17-86

Margaret E. Brower, Ph.D.
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Date: Oct. 16, 1986

Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irving Mauer
Date: 10/20/86

Jane Harris, Ph.D.
EPA Section Head

Signature: Jane E Harris
Date: 10/20/86

005425

DATA EVALUATION REPORT

MRID NO.: 080714

STUDY TYPE: Chronic toxicity feeding study in dogs.

ACCESSION NUMBER: Not available.

TEST MATERIAL: Dithane M-45.

SYNONYMS: Mancozeb.

STUDY NUMBER(S): Not available.

SPONSOR: Rohm and Haas Company, Philadelphia, PA.

TESTING FACILITY: Department of Pharmacology, Medical College of Virginia.

TITLE OF REPORT: Toxicologic Study on the Effect of Adding Dithane M-45 to the Diet of Beagle Dogs for a Period of Two Years.

AUTHOR(S): Larson, P. G., Borzelleca, J. F., and Ambrose, A. M.

REPORT ISSUED: December 1, 1965.

CONCLUSIONS:

When fed to beagle dogs for 24 months at levels of 25, 200, or 1000 ppm, Dithane M-45 produced slight but significant decreases at 24 months in the uptake of radioiodine [^{131}I] in low- and mid-dose females sampled at 48 hours (88 and 86 percent of controls, respectively) and at all doses at 72 hours (84, 81, and 83 percent of control in low-, mid- and high-doses, respectively); however, all values were within the normal range for dogs (14-40 percent). No changes in [^{131}I] uptake were found in dosed males, and PBI values were similar in all groups. Two high-dose animals (one male, one female) showed grade 1 hyperplasia of the thyroid; focal hyperplasia was seen in one mid-dose female. Absolute and relative weights of thyroids were increased ($p < 0.05$) only in mid-dose females. Testes in all male groups (control, 1/4; low-, 1/4; mid-, 3/4; and high-dose, 2/4) showed age-related incidences of minimal atrophy.

Based upon thyroid hyperplasia at the HDT, a NOEL of 100 ppm (estimated intake 2.5 mg/kg/day) for systemic toxicity has been assigned.

Classification: Core Supplementary.

A. MATERIALS:

1. Test Compound: Dithane M-45. Description: solid. Batch No.: 16-3202. Purity: 86.2 percent.

2. Test Animals: Species: dog. Strain: purebred beagle. Age: about 6 months. Weight: males, 5.3-8.5 kg; females, 4.9-7.2 kg. Source: not reported.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test groups:

Test group	Dose in diet (ppm)	Main study (24 weeks) Males	Main study (24 weeks) Females
1 Control	0	4	4
2 Low (LDT)	25	4	4
3 Mid (MDT)	100	4	4
4 High (HDT)	1000	4	4

2. Diet Preparation: A single [illegible] refrigerated test material was used for 6 months and [illegible] ced. Dithane M-45 was suspended in a 12 percent Mazola [illegible] nt cod liver oil mixture and added to the basal diet [illegible] to feeding time. Details regarding the frequency of preparation and storage conditions for the diets were not reported.

   Results: The results from analyses of concentrations, homogeneity, and stability of the test compound in the diets were not reported. Dietary concentrations of Dithane M-45 were corrected to the 100 percent purity of the active ingredient; however, method of analysis and data were not given in the report.

3. Animals received food and water ad libitum.

4. Statistics: The methodology of statistical evaluation was not reported.

C. METHODS AND RESULTS:

1. Observations: Prior to study initiation, all dogs were treated for intestinal parasites and immunized against distemper, infectious hepatitis, and leptospirosis.

   Details about routine animal examinations were not provided in this report.

   Results: According to study authors, no compound-related signs of toxicity were observed. No deaths occurred during the study period.

2. Body Weight: Body weights were determined prior to initiation of study and at weeks 1, 3, 6, 13, 26, 52, 78, and 104.

   Results: Mean body weight data are presented in Table [illegible]. No significant differences were seen between any dose group of males or females and their respective controls, except for the 1000 ppm male group which showed a 60 percent gain over the controls, statistically significant ($p < 0.05$). No compound-related trends were observed.

3. Food consumption, water consumption, and compound intake: Food consumption was determined and mean daily food intake was calculated.

   Results: No significant intergroup differences in food consumption were found. Food efficiency and compound intake were not determined.

4. Ophthalmological examinations were not performed.

TABLE 1. Mean Body Weight (kg ± SD) for Dogs Fed Dithane M-45 for 104 Weeks[a]

Dose (ppm)	Week on Test 0	13	52	104	Wt. Gained ± SD
			Males		
Control	6.80±0.80	9.33±0.61	10.25±0.45	11.00±1.10	4.20±1.10
25	6.88±1.37	9.43±1.14	10.73±1.61	11.25±2.40	4.38±1.11
100	6.80±1.11	9.83±0.99	11.78±0.81	13.53±2.05	6.73±1.40*
1000	7.15±1.07	9.65±1.20	10.63±1.09	11.10±1.44	3.95±1.39
			Females		
Control	5.60±0.58	6.48±0.95	7.45±1.01	7.85±1.66	2.25±1.60
25	5.98±0.83	7.83±1.42	8.95±1.19	9.20±1.24	3.23±1.24
100	5.85±0.70	7.53±0.69	8.98±0.91	9.58±0.40	3.73±0.39
1000	5.93±0.81	7.58±0.67	8.98±1.02	9.43±1.75	3.50±1.60

[a]Mean values based on four animals per dose group.

*Significantly different from control values by ANOVA followed by Duncan's test for multiple comparisons ($p < 0.05$). Statistical analysis was conducted by our statistician.

5. Blood was collected for hematology from four animals/sex/dose before treatment and at 3, 6, 12, 18, and 24 months. The CHECKED (X) parameters listed below were examined.

Protein-bound iodine (PBI) and uptake of ([^{131}I]) by the thyroid were determined on control and high-dose dogs at months 6 and 12 and on all groups of males and females at 24 months. In addition, at month 24, bromosulphalein (BSP), serum glutamate-oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), blood urea nitrogen (BUN), and blood glucose were determined in all dogs.

a. Hematology

X	Hematocrit (HCT)†		Total plasma protein (TP)
X	Hemoglobin (HGB)†	X	Leukocyte differential count
X	Leukocyte count (WBC)†		Mean corpuscular HGB (MCH)
	Erythrocyte count (RBC)†		Mean corpuscular HGB concentration (MCHC)
	Platelet count†		Mean corpuscular volume (MCV)
			Reticulocyte count

Results: Examination of the mean values indicated no dose-related trends. Changes in parameters that reached a level of significance in dosed dogs ($p < 0.05$) were sporadic and the values were within the normal range of biological variation for beagle dogs (Table 2). At month 12, mid-dose males showed a significant ($p < 0.05$) decrease in hematocrit (13 percent) and hemoglobin (11 percent) and an increase ($p < 0.05$) in leukocyte count (30 percent). At month 24, percent neutrophils were increased 15 percent and lymphocytes were decreased 30 percent in high-dose males when compared to controls. Mid-dose females showed a 43 and 36 percent decrease in lymphocytes ($p < 0.05$) at 6 and 24 months, respectively; and a 23 percent increase ($p < 0.05$) in neutrophils at 6 months.

b. Clinical Chemistry

	Electrolytes		Other
	Calcium†		Albumin†
	Chloride†		Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
	Phosphorus†		Cholesterol†
	Potassium†		Globulins
	Sodium†	X	Glucose†
	Enzymes		Total bilirubin†

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 2. Selective Group Mean Hematologic Values (±SD) in Dogs Fed Dithane M-45 for 24 Months[a]

	Month								
	6			12			24		
Dose (ppm):	0	100	1000	0	100	1000	0	100	1000
Males									
Hematocrit (%)	45.0 ±3.4	43.75 ±3.5	44.75 ±2.5	49.25 ±3.8	42.75* ±1.7	49.25 ±3.0	48.0 ±1.4	46.75 ±1.0	49.50 ±1.0
Hb (g/100 mL)	15.6 ±0.8	14.5 ±0.2	16.08 ±1.0	15.75 ±1.1	13.98* ±0.40	16.2 ±1.0	14.65 ±0.80	14.6 ±0.2	14.95 ±0.90
WBC (x 10^3)	21.93 ±1.7	21.80 ±6.6	23.88 ±6.30	18.53 ±2.0	24.13* ±1.8	16.38 ±1.5	20.35 ±4.1	20.05 ±5.1	16.0 ±1.30
Lymphocyte (%)	27.5 ±5.4	20.25 ±5.3	29.75 ±4.0	24.5 ±4.1	17.25 ±9.2	28.25 ±4.9	28.75 ±5.1	24.25 ±4.6	20.25* ±3.8
Neutrophils (%)	69.50 ±5.0	74.75 ±4.9	67.5 ±7.0	73.0 ±1.4	76.5 ±9.7	66.0 ±6.5	68.5 ±4.8	72.25 ±4.1	78.5* ±4.0
Females									
Hematocrit (%)	51.0 ±4.24	51.25 ±4.90	52.75 ±3.60	52.25 ±1.90	51.5 ±1.0	52.0 ±2.2	50.5 ±1.9	51.25 ±4.30	49.75 ±2.80
Hb (g/100 mL)	16.38 ±1.20	16.0 ±0.3	17.13 ±0.40	16.9 ±0.7	17.13 ±0.4	16.85 ±0.7	16.83 ±1.60	15.85 ±1.50	15.3 ±0.8
WBC (x 10^3)	20.88 ±4.30	27.48 ±14.80	18.58 ±5.40	15.45 ±2.6	15.88 ±0.8	16.45 ±2.50	16.93 ±4.9	19.35 ±1.6	15.35 ±2.6
Lymphocyte (%)	31.0 ±1.8	17.75* ±6.5	25.25 ±0.5	17.75 ±5.7	25.0 ±6.6	25.0 ±6.1	28.5 ±6.2	18.25* ±2.8	29.75 ±5.6
Neutrophils (%)	64.75 ±1.9	79.5* ±7.0	70.5 ±3.1	66.75 ±8.0	79.0 ±5.0	71.0 ±6.7	70.0 ±5.9	81.25 ±2.8	69.5 ±5.3

[a]Mean values based on four dogs/group.

*Significantly different from control by ANOVA, followed by Duncan's test for multiple comparisons ($p < 0.05$). Statistical analyses were conducted by our statistician.

X	Alkaline phosphatase (ALP)		Indirect bilirubin
	Cholinesterase		Total protein†
	Creatinine phosphokinase†		Protein quotient (A/G ratio)
	Lactic acid dehydrogenase		Triglycerides
	Serum alanine aminotransferase (also SGPT)†	X	Serum protein-bound iodine (PBI)
		X	Thyroid radioiodine ([^{131}I])
X	Serum aspartate aminotransferase (also SGOT)†	X	Bromosulphalein retention

Results: It was reported that levels of glucose, SGOT, ALP, BUN, and findings on bromosulphalein retention were comparable to control values in all dosed groups at 24 months. Mean values were presented that combined data for males and females. When data were separated by sex and statistically analyzed by our reviewers, the only significant change ($p < 0.05$) was a decrease in glucose in females receiving 25 ppm Dithane M-45. Values in females receiving 100 or 1000 ppm were comparable to controls.

In males, values for PBI were similar in control and high-dose groups at 6 and 12 months and were similar in all dosed groups and controls at 24 months. The uptake of [^{131}I] was similar in dose and control groups at 6 and 24 months; at the 12-month interval it was slightly but significantly ($p < 0.05$) higher than control in high-dose males at the 24-hour sampling period but not at 48 or 72 hours. In females, PBI and [^{131}I] uptake were similar in controls and high-dose groups at 6 and 12 months. At month 24, low- and mid-dose females showed a significant ($p < 0.05$) decrease (12 and 14 percent, respectively) in [^{131}I] uptake at 48 hours (Table 3). At 72 hours, these values were further decreased to 16 and 19 percent, respectively. In addition, high-dose females also showed a significant ($p < 0.05$) decrease of 17 percent in [^{131}I] uptake. All values, however, are within the normal range for Beagle dogs (15-40 percent).

6. Urinalyses: Urine was collected from animals at 3, 6, 12, and 18 months. The CHECKED (X) parameters were examined.

	Appearance†	X	Glucose†
	Volume†	X	Ketones†
	Specific gravity†		Bilirubin†
X	pH		Blood†
	Sediment (microscopic)†		Nitrate
X	Protein†		Urobilinogen

Results: No significant differences between the controls and dosed groups were found in any of the parameters determined.

7. Sacrifice and Pathology: All animals were sacrificed on schedule and were subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

TABLE 3. Group Mean Values (±SD) for Protein-Bound Iodine (PBI) and [^{131}I] Uptake in Beagle Dogs Fed Dithane M-45 for 24 Months[a]

Dose (ppm)	PBI (mcg %)	Percent [^{131}I] Uptake		
		24 Hr	48 Hr	72 Hr
		Males		
0	5.7±0.9	21.57±4.0	31.10±5.6	32.73±5.5
25	4.9±0.9	26.83±3.1	29.05±4.4	30.94±4.0
100	4.93±0.8	23.79±3.5	27.68±3.7	28.16±4.5
1000	5.48±0.8	25.47±6.1	28.19±7.6	26.1±8.3
		Females		
0	6.15±0.9	26.2 ±2.00	32.70±2.30	34.94±3.70
25	5.55±0.6	23.16±1.53	28.80±2.40*	29.36±2.40*
100	5.30±0.6	22.43±0.80	28.05±0.80*	28.30±1.00*
1000	5.60±0.9	25.79±3.30	30.34±2.20	28.94±2.50*

[a]Mean values based on four dogs/group.

*Significantly different from control by ANOVA, followed by Duncan's test for multiple comparisons ($p < 0.05$). All calculations and statistical analyses were performed by our statistician.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta†	X	Brain†
	Salivary glands†	XX	Heart†		Peripheral nerves†
	Esophagus†		Bone marrow†		Spinal cord (3 level)
X	Stomach†		Lymph nodes†		Pituitary†
X	Duodenum†	XX	Spleen†		Eyes (optic nerve)†
X	Jejunum†		Thymus†		Glandular
X	Ileum†		Urogenital	XX	Adrenals†
X	Cecum†	XX	Kidneys†		Lacrimal gland
X	Colon†	X	Urinary bladder†		Mammary gland†
X	Rectum†	XX	Testes†		Parathyroids†
XX	Liver†		Epididymides	XX	Thyroids†
	Gall bladder†		Prostate		Other
X	Pancreas†		Seminal vesicle	X	Bone (femur)†
	Respiratory	X	Ovaries	X	Skeletal muscle†
	Trachea†		Uterus		Skin
X	Lung†				All gross lesions and masses

Because of a compound-related decrease in [^{131}I] uptake, major efforts were made to study the histopathologic lesions in the thyroid glands. Two sections from each thyroid lobe of every animal were examined. Alcian Blue-PAS stain was used to estimate the amount of mucosubstances in the follicles.

Results:

a. Organ Weights: There were some elevations in organ weights in dogs dosed with Dithane M-45 (Table 4). Mean absolute heart weights in low- and mid-dose females were significantly ($p < 0.05$) increased by 25 and 28 percent, respectively. Mid-dose females also showed 54 and 64 percent increases in mean absolute weights for spleen and thyroid, respectively. Mid-dose males showed 15 and 52 percent increases in heart and kidney weights, respectively. Organ-to-body weight ratios of spleen and thyroid in mid-dose females were increased by 31 and 40 percent; increases in heart and liver weights were not accompanied by corresponding increases in organ-to-body weight ratios.

No other statistically significant changes in mean absolute or relative weights were seen.

According to the study authors, elevated organ weights seen in some dosed animals were due to increased body weights, and no dose- or compound-related trend was present. However, individual organ weight data were not included in the CBI report to evaluate and verify these statements.

TABLE 4. Mean Organ Weights (g±SD) and Organ-to-Body Weight Ratios (g/kg) in Dogs Fed Dithane M-45 for 24 Months[a]

Dose (ppm)	Body Weight (kg)	Heart	Spleen	Kidneys	Liver	Thyroid
			Females			
Control	8.0±1.2	60.6±11.2 (7.55±0.29)	50.9± 8.6 (6.42±1.19)	40.5± 9.2 (5.03±0.60)	235±31.0 (29.51±3.04)	0.39±0.09 (0.049±0.01)
25	9.3±1.3	75.7± 4.9* (8.30±1.17)	53.5±25.8 (6.00±3.16)	43.1± 2.4 (4.74±0.85)	250±29.0 (27.28±3.51)	0.63±0.21 (0.067±0.02)
100	9.3±0.5	77.3± 5.6* (8.31±0.37)	78.5± 5.2* (8.44±0.44*)	43.0± 4.4 (4.63±0.53)	252±13.0 (27.08±1.45)	0.64±0.14* (0.069±0.01)*
1000	9.6±1.7	72.7±11.6 (7.65±0.25)	60.0±14.4 (6.36±1.57)	45.9± 7.9 (4.85±0.86)	329±37.0* (34.80±4.38)	0.46±0.07 (0.050±0.01)
			Males			
Control	11.2±1.6	80.7± 2.4 (7.24±0.80)	56.2±21.0 (5.06±2.01)	47.2± 5.0 (4.22±0.37)	324±43 (28.83±1.82)	0.67±0.07 (0.060±0.01)
25	11.4±2.4	89.6±16.5 (7.91±0.54)	64.9±20.6 (5.66±1.48)	53.6± 6.9 (4.80±0.73)	298±36 (26.63±3.26)	0.65±0.08 (0.058±0.01)
100	13.4±1.4	93.1± 8.9* (6.98±0.46)	74.6±32.9 (5.52±1.98)	65.4± 9.9* (4.97±1.28)	385±23 (27.11±6.33)	0.73±0.18 (0.053±0.01)
1000	11.2±1.2	89.1±10.2 (8.08±1.46)	78.5± 6.3 (7.08±0.72)	52.4± 8.6 (4.77±1.18)	355±70 (31.98±5.21)	0.68±0.13 (0.061±0.01)

[a]The values in parentheses are organ-to-body weight ratios in g/kg.

*Value is significantly different from control ($p < 0.05$).

b. Gross Pathology: Gross pathologic findings were not reported.

c. Microscopic Pathology (Nonneoplastic): According to the study authors, no major histopathologic lesions attributable to Dithane M-45 administration were observed. One high-dose animal of each sex showed grade 1 hyperplasia of the thyroid; focal hyperplasia of the thyroid was observed in one mid-dose female. However, no dose-related changes in the thyroid follicle morphology were observed. Alcian Blue-PAS stain also showed no significant intergroup differences in the amount of mucosubstances present in the follicles. No detailed description of grade 1 hyperplasia was given.

Testes in all male treatment groups (control 1/4; low-dose, 1/4; mid-dose, 3/4; high-dose, 2/4) showed incidences of minimal atrophy. Focal hemorrhage in the lung was observed in one high-dose female. No distinct lesions that could be related to the increased heart, spleen, kidney, and liver weights were observed.

No neoplastic lesions were observed.

D. DISCUSSION:

The experimental design was incomplete and inadequate to assess the systemic chronic toxicity of Dithane M-45. In a number of summary tables, data for males and females were combined, and some summary tables were not supported by the individual animal data. Under the conditions of the study, compound-related biochemical aberrations and histopathologic lesions were restricted to the female thyroids. At month 24, low- and mid-dose females showed a significant ($p < 0.05$) decrease (12 and 14 percent, respectively) in uptake of [^{131}I] at 48 hours. At 72 hours, these values were further decreased to 16 and 19 percent, respectively. At 72 hours, high-dose females showed a significant ($p < 0.05$) decrease of 17 percent in [^{131}I] uptake. However, all values are within the normal range for Beagle dogs.[1] Only one high-dose animal of each sex showed grade 1 hyperplasia of the thyroid; focal hyperplasia of thyroid was observed in one mid-dose female. Absolute and thyroid-to-body weight ratios were significantly ($p < 0.05$) increased only in the mid-dose females. Although [^{131}I] uptake was significantly reduced at all dose levels in females, this could not be correlated with any other thyroid effects except for the definitive hyperplasia at the HDT.

Testes in all male groups (control, 1/4; low-dose, 1/4; mid-dose, 3/4; high-dose, 2/4) showed age-related incidences of minimal atrophy.

There were no toxicologically important effects on mortality, body weights, food consumption, and hematology.

[1] Lombardy, M. H., Comar, C. L. and Kirk, R. W. Diagnosis of thyroid gland function in the dog. Am. J. Vet. Res. 23:412-420, 1962.

Some major deficiencies of this study are as follows:

1. Concentration, homogeneity, and stability of the test compound in the diets were not determined.

2. No statistical procedures or names of methods used were given in the report.

3. Ophthalmologic examinations were not conducted.

4. In many summary tables, male and female values were combined to calculate the means.

5. Individual organ weight data were not provided in the report.

6. The number of organs weighed and examined histopathologically was insufficient.

7. Not all of the required hematologic and clinical chemistry parameters were determined.

8. Thyroid function tests (T3, T4, or TSH) were not conducted.

Hence, the study is graded Core Supplementary Data according to FIFRA Guidelines. The LOEL is 1000 ppm (estimated intake, 25 mg/kg/day) based on thyroid hyperplasia and the NOEL for systemic toxicity is 100 ppm (estimated intake, 2.5 mg/kg/day).

I. DATA EVALUATION

REPORTS

EPA: 68-02-4225 005425
DYNAMAC No. 1-009E
June 4, 1986

DATA EVALUATION RECORD

MANCOZEB

Subchronic Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Subchronic inhalation study in rats--thirteen-week interim report. (Unpublished study No. 86R-003 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261539.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 6-4-86

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005425

1. CHEMICAL: Mancozeb; dithane M-45®; Manzate 200®; coordination product of zinc icn and manganese ethylenebisdithiocarbamate; $C_4H_6N_2S_4MnZn$.

2. TEST MATERIAL: Mancozeb (lot No. 4339; TD No. 85-015; product code 6-2804) was described as a yellow powder containing 83.35 percent active ingredient.

3. STUDY/ACTION TYPE: Subchronic inhalation toxicity study in rats.

4. STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Subchronic inhalation study in rats--thirteen-week interim report. (Unpublished study No. 86R-003 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261539.

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7. CONCLUSIONS:

A. Groups of 38 male and 38 female rats were exposed to mancozeb target concentrations of 0, 20, 80, or 320 mg/m^3 for 13 weeks. Groups of five males and five females were sacrificed after 4 weeks of exposure, and groups of 16 males and 16 females were necropsied at the end of the 13 week exposure. In addition, 17 males and 17 females were held for an additional 13 weeks following the exposure phase of the study as a recovery study. [The results of the recovery study, were not included in this report.]. The actual mean respirable concentrations to which the rats were exposed were 0, 8, 36, and 144 mg/m^3, respectively. The male rats exposed to 144 mg/m^3 exhibited significant ($p \leq 0.05$) reduced mean body weight and body weight gain for most of the exposure period, whereas no such effects were noted in female rats. Mean corpuscular volume, mean corpuscular hemoglobin concentration, serum triglyceride levels, and inorganic phosphorus levels were significantly ($p \leq 0.05$) altered; however, they were within normal ranges for rats and were not considered of biological relevance. Thyroid function tests revealed significantly reduced T4 serum levels in female rats exposed to 144 mg/m^3 for 13 weeks. In samples collected at the termination of exposures, blood, urine, and thyroid samples exhibited an exposure-response increase in ethylenethiourea (ETU) and ethylenebisdithiocarbamate (EBDC) concentrations. These data support the hypothesis that mancozeb is metabolized to ethylene-thiourea. Organ weight changes included reduced kidneys and heart weights in male rats exposed to 144 mg/m^3; this may have reflected the reduced body weight of these animals. No remarkable ophthalmologic findings were reported. Among the histologic findings, hyperplasia of the follicular epithelium was noted in 3 of 10 females exposed to 144 mg/m^3, yellow-brown granular pigment in kidneys of both males and females exposed to 36 or 144 mg/m^3, and several lesions in the respiratory tract. The thyroid changes were related to exposure to mancozeb whereas the respiratory tract lesions are typically observed following exposure to dusts. The respiratory tract lesions may be indicative of a progressive disease. The authors considered renal inclusions to represent the elimination of a urinary metabolite. However, the granular form of the pigmented material may be indicative of progressive disease or lead to chronic lesions following the inhalation of mancozeb. Based on the renal inclusions, the LOAEL is 36 mg/m^3 and the NOAEL is 8 mg/m^3 for rats exposed to mancozeb dust aerosols via nose-only exposure.

Item 8--see footnote 1.

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[1] Only items appropriate to this DER have been included.

9. BACKGROUND: The exposure levels selected for this subchronic study were derived from a 2-week inhalation study designed to determine exposure levels and the mode of exposure. Nose-only was selected over whole body exposure. From the 2-week study, the NOAEL was 55 mg/m^3 and the LOAEL was 258 mg/m^3 for rats exposed to mancozeb for 10 exposures based on the respirable concentrations of mancozeb in the chambers.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, mancozeb, contained 83.35 percent active ingredient, which was a coordination product of zinc ion and manganese ethylenebisdithiocarbamate. The exposure concentrations used in this study were based on the formulated test material, as received. Four groups of animals, designated 1, 2, 3, and 4, were exposed to target aerosol concentrations of mancozeb of 0, 20, 80, or 320 mg/m^3. Animals were exposed (nose-only) 6 hours a day, 5 days a week.

2. Thirty-eight male and 38 female Crl:CD(SD)BR rats (Charles River-Lakeview, Newfield, NJ) were randomly assigned to each of the four exposure groups. The rats were 35 days old at the initiation of the study and weighed between 157 and 214 g (males) and 124 and 168 g (females). Due to an error in sexing, group 2 contained 37 males and 39 females. The animals in each of the four groups were further divided into three subgroups, designated A, B, and C. Animals from subgroup A were necropsied after 4 weeks, and subgroup B animals were necropsied after 13 weeks of exposure. Subgroup C animals were to be necropsied after a 13-week post-exposure recovery period; data for the recovery period will appear in the final report.

3. The animals were housed individually in stainless steel wire-mesh cages in an environmentally controlled room while they were not in the exposure chambers. The room was maintained at a temperature ranging between 70 and 80°F (21-27°C), a relative humidity of 30-70 percent, and a 12 hour light, 12 hour dark cycle. During exposure, the exposure chambers were maintained under similar conditions. Animals were provided food and water ad libitum except during exposure and during the pre-necropsy fasting period. During exposure, animals were housed in individual PVC nose-only restraining tubes which were attached to the front of the exposure chambers.

4. The powdered test material was placed in a sample reservoir, and was forced through the funnel-shaped bottom of the reservoir by a plunger, into a conducting duct which contained a blow-jet nozzle. From the conducting duct, the test material was incorporated into an air stream which entered the air-mixing turret of a stainless steel and glass exposure chamber. The configuration and stroke frequency of the plunger were varied in order to achieve the different exposure concentrations. The exposure chambers were supplied with filtered room air, and the chamber air flow rate was monitored. The aerosol concentrations in each exposure chamber were also monitored periodically each day in order to check and adjust the aerosol output of the dust generators. The actual chamber analytical concentrations and the particle size distributions were determined gravimetrically. Samples were taken daily from all four chambers to determine chamber analytical concentrations. Particle size samples were taken weekly from chambers 2, 3, and 4, which housed groups 2, 3, and 4, respectively; particle size samples were not taken from chamber 1 (control chamber). The exposure concentrations cited in the report were the respirable dust concentrations, which were calculated from the total analytical dust concentrations and the respirable fraction. The respirable fraction was calculated from the mass median diameter and the geometric standard deviation. Temperature and humidity were monitored continuously in the chambers and in the animal holding room.

5. All animals were examined and weighed the day before the first exposure (week 0), and then weekly until study termination. Animals were examined before, during, and after each exposure for signs of toxicity and mortality. Each animal was given an ophthalmologic examination prior to the first exposure and again during week 12. At the scheduled intervals, after 4 weeks and after 13 weeks of exposure, animals were sacrificed and necropsied. Blood samples were drawn from all animals scheduled for histopathologic evaluation. These samples were used to evaluate eight hematologic and 15 clinical chemistry parameters. Thyroid functions (T3, T4, and TSH serum levels) were also evaluated.

   At necropsy, all animals were examined macroscopically. After 4 weeks of exposure, the following target organs from all animals sacrificed were removed, fixed in formalin, and histologically examined: lungs; lymph node (peribronchial); nasal turbinates; trachea; and thyroid/parathyroid. Absolute organ weights were recorded and relative organ weights (organ-to-body weight ratios) were determined for lungs and thyroid/parathyroid. After 13 weeks of exposure, the absolute and relative weights of 10 organs from all sacrificed animals were recorded. Tissues from the above-mentioned target organs, as well as liver and kidney tissues and all gross lesions and masses were histologically

examined for rats exposed to 20 or 80 mg/m^3. A complete histopathological examination was performed on rats exposed to 0 or 320 mg/m^3, which included tissues of 40 organs and all gross lesions and masses.

Six male and six female rats from each group had previously been designated for residue analysis, and were not sacrificed at study termination. After 13 weeks of exposure, these rats were placed in metabolism cages for 24 hours, during which time the total urine output was collected and frozen. After the 24-hour period, these animals were sacrificed, and the lungs, trachea, and livers were removed and placed in frozen storage. The blood and thyroids were also removed and frozen; the frozen samples and urine were sent to Enviro-Bio-Tech, Ltd. (Bernville, PA) for determination of residual levels of ETU and EBDC as carbon disulfide.

6. The data for body weights, body weight changes, hematologic parameters, clinical chemistry parameters, thyroid function parameters, organ weights, and organ-to-body weight ratios were evaluated using appropriate statistical methods. A difference between exposure groups and controls was considered statistically significant at $p \leq 0.05$.

B. Protocol: The study protocol is included in Appendix A.

12. REPORTED RESULTS:

A. Chamber Conditions: It was reported that the air flow rate during exposure in all chambers was 400 L/min., which resulted in a 99 percent aerosol equilibrium time (t99) of 14.4 min. or 4.0 percent of the exposure time. The chamber concentration and aerosol characterization data are shown in Table 1.

1. 4 Weeks of Exposure: The mean analytical aerosol concentrations of mancozeb in the chambers were found to be 0 (group 1), 22 (group 2), 86 (group 3), and 308 (group 4) mg/m^3; these values corresponded to target concentrations of 0, 20, 80, and 320 mg/m^3. The corresponding respirable concentrations were 0, 8, 40, and 127 mg/m^3 for groups 1, 2, 3, and 4, respectively. The mean mass median diameter (MMD) of the aerosol particles ranged from 3.7 to 4.4 micrometers, the mean geometric standard deviation (GSD) ranged from 2.1 to 2.3, and the respirable fraction ranged from 42 to 47 percent. The mean temperature in the chambers ranged from 20.0 to 21.8°C, and the mean relative humidities ranged from 71 to 74 percent.

2. 13 Weeks of Exposure: The mean analytical aerosol concentrations of mancozeb were 0, 18, 79, and 326 mg/m^3; these values corresponded to target concentrations of 0, 20, 80, and 320 mg/m^3 respectively. The corresponding respirable

TABLE 1. Chamber Concentration and Aerosol Characterization

Target Exposure Concentration (mg/m^3)	Mean Analytical Exposure Concentration (mg/m^3)	Range of Daily Exposure Concentration (mg/m^3)	Respirable Exposure Concentration (mg/m^3)	Mean Mass Median Diameter (micrometers)	Geometric Standard Deviation	Respirable Fraction (percent)
After 4 Weeks						
0	0	0	0	0	0	0
20	22	1- 70	8	4.1	2.1	43
80	86	36-152	40	3.7	2.2	47
320	308	161-572	127	4.4	2.3	42
After 13 Weeks						
0	0	0	0	0	0	0
20	18	7- 30	8	3.9	2.1	45
80	79	47-117	36	3.8	2.1	46
320	326	215-514	144	4.2	2.1	42

concentrations were 0, 8, 36, and 144 mg/m^3, respectively. The aerosol particles had mean MMD of 3.8 to 4.2 micrometers, mean GSD of 2.1, and respirable fraction ranged from 42 to 46 percent. The mean chamber temperatures ranged from 19.6 to 21.1°C, and mean relative humidities ranged from 68 to 77 percent.

B. Clinical Observations:

1. 4 Weeks of Exposure: Six animals, distributed among the four groups, died during this study period. The authors attributed these deaths to asphyxiation caused by excessive restraint in the nose-only restraining tubes. Alopecia and missing tail tips were noted for several animals; these findings were attributed to injury by the restraining tubes.

2. 13 Weeks of Exposure: During week 5 to week 13, five additional deaths occurred, two of these were attributed to excessive restraint. One female, exposed to 80 mg/m^3, died as the result of gastric torsion. One male exposed to 320 mg/m^3 died with a prostate abscess. One male exposed to 20 mg/m^3 exhibited gasping, rales, bradypnea, dyspnea, a red serosanguinous exudate on the muzzle, and bright red spotting on the dropping sheet during week 5. The animal appeared emaciated through week 11 with misaligned incisors at week 7, and died during exposure in week 12 with a nasal abscess around a tooth. Several animals exhibited alopecia, dark brown staining of the fur, abrasions, missing tail tips, wet abdominal fur, and/or red exudate around the eyes, all of which were attributed to the restraining method.

C. Body Weights and Body Weight Gains: A summary of the mean body weight data is presented in Table 2.

1. 4 Weeks of Exposure: Mean body weights and mean body weight gains of males exposed to 320 mg/m^3 were significantly reduced ($p \leq 0.05$) compared to controls during weeks 2 through 4. No other exposure-related body weight effects were noted during the first 4 weeks of exposure.

2. 13 Weeks of Exposure: Males exposed to 320 mg/m^3 exhibited significantly reduced ($p \leq 0.05$) mean body weights and mean body weight gains compared to controls during weeks 7 through 13. Females exposed to 20 mg/m^3 exhibited a significantly increased ($p \leq 0.05$) body weight gain as compared to controls. No other exposure-related body weight effects were noted during this time period.

D. Hematology:

1. 4 Weeks of Exposure: An insufficient amount of blood was obtained from 11 of 20 females at the 4-week necropsy interval. The data, therefore, were insufficient to make a meaningful statistical evaluation of female hematologic

TABLE 2. Selected Mean Body Weights (± SD) and Total Body Weight Gain (± SD) in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Group Mean Body Weight (g) at Week				Total Body Weight Gain (Week 0-13)[a]
	0	4	8	13	
Males					
0	182.97 ±12.67	336.4 ±30.0	399.3 ±30.2	360.3 ±40.6	277.1 ±34.0
20	180.27 ±9.83	327.2 ±30.0	383.2 ±40.7	440.1 ±50.6	259.6 ±45.8
80	182.45 ±11.79	329.7 ±22.9	393.3 ±32.1	451.9 ±40.7	271.0 ±37.1
320	181.37 ±10.64	318.0* ±23.3	376.8* ±29.5	429.0* ±34.8	248.3* ±30.5
Females					
0	145.05 ±9.19	200.1 ±17.5	223.0 ±16.3	241.1 ±18.3	95.7 ±16.8
20	142.49 ±9.56	203.3 ±18.3	228.5 ±19.4	251.9 ±25.8	110.1* ±20.1
80	143.92 ±6.19	197.1 ±12.9	225.2 ±14.0	241.6 ±17.4	98.5 ±14.8
320	146.13 ±9.86	205.4 ±14.7	228.4 ±15.7	245.9 ±18.3	99.5 ±14.6

[a]Total weight gain for rats alive at week 13.

*Significantly different from control value ($p \leq 0.05$).

parameters. No hematologic effects were seen for males in any exposure group.

2. 13 Weeks of Exposure: Females exposed to 320 mg/m^3 exhibited a significant increase ($p \leq 0.05$) in mean corpuscular volume (MCV) and a significant decrease ($p \leq 0.05$) in mean corpuscular hemoglobin concentration (MCHC) compared to controls. However, the parameters from which the MCV and MCHC values were derived (hematocrit, red blood cell count, and hemoglobin) were not affected. Therefore, these differences were not considered toxicologically relevant. No other hematologic effects were seen in males or females. Table 3 summarizes mean values of selected hematologic parameters for females.

E. Clinical Chemistry:

1. 4 Weeks of Exposure: No effects were seen in clinical chemistry parameters for males or females in any group after 4 weeks of exposure.

2. 13 Weeks of Exposure: A significant reduction ($p \leq 0.05$) was seen in triglyceride levels in males exposed to 320 mg/m^3 after 13 weeks of exposure. The authors considered this effect to be related to the reduced body weights which were seen these males, and was, therefore, a secondary effect of mancozeb exposure.

Inorganic phosphorus was significantly reduced ($p \leq 0.05$) in females exposed to 80 mg/m^3 compared to controls. This effect was not observed in females exposed to 320 mg/m^3, and was, therefore, not considered toxicologically relevant by the authors. No other clinical chemistry effects were seen in males or females.

F. Thyroid Function Data:

1. 4 Weeks of Exposure: T3, T4, and TSH serum levels were not affected in males or females of any group during the first 4 weeks of exposure.

2. 13 Weeks Exposure: A significant reduction ($p \leq 0.05$) in T4 serum level was noted in females exposed to 320 mg/m^3; this was considered to be exposure related. No other effects on thyroid function were noted. Female T4 serum levels are presented in Table 4.

G. Residue Analysis (conducted by subcontractor and included in Appendix of report): Urine, blood, and thyroid samples collected after 13 weeks of exposure were analyzed for ETU and EBDC; the data are presented in Table 5. The subcontractor reported that EDBC residues increased in urine at 20, 80, anc 320 mg/m^3 and in blood at 320 mg/m^3. The ETU residues increased in urine and blood at 20, 80, and 320 mg/m^3 and in thyroid at 80 and 320 mg/m^3.

TABLE 3. Selected Mean Hematology Values (± SD) in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Parameter/Group Mean Value After 13 Weeks				
	RBC ($10E6/mm^3$)	HCT (%)	HGB (g/100 mL)	MCV (μm^3)	MCHC (%)
Males					
0	8.63 ±0.45	50.4 ±2.0	14.4 ±0.6	58 ±2.0	28.6 ±0.4
20	8.38 ±0.41	49.3 ±0.9	14.1 ±0.7	59 ±2.0	28.5 ±0.8
80	8.47 ±0.34	49.9 ±1.4	14.1 ±0.4	59 ±2.0	28.4 ±0.4
320	8.49 ±0.40	50.0 ±2.9	14.4 ±0.7	59 ±2.0	28.7 ±0.7
Females					
0	7.95 ±0.27	47.9 ±1.7	13.8 ±0.4	60 ±1.0	28.9 ±0.5
20	8.12 ±0.39	49.6 ±2.6	14.1 ±0.7	61 ±1.0	28.5 ±0.4
80	8.09 ±0.40	49.9 ±1.5	14.1 ±0.6	62 ±2.0	28.3 ±0.8
320	7.81 ±0.52	48.5 ±3.6	13.6 ±0.9	62* ±1.0	28.0* ±0.6

*Significantly different from control value ($p \leq 0.05$).

TABLE 4. Selected Thyroid Function Data for Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Group Mean (± SD) T4 Serum Level (µg/dl)	
	After 4 Weeks	After 13 Weeks
Males		
0	4.61 ±1.22	4.39 ±0.76
20	5.40 ±0.75	4.17 ±0.31
80	4.71 ±0.59	4.50 ±0.67
320	4.13 ±1.11	4.02 ±0.60
Females		
0	3.59 ±0.77	3.17 ±0.60
20	32.6 ±1.13	3.11 ±0.76
80	3.29 ±0.63	2.77 ±0.75
320	2.71 ±0.54	2.18*±0.74

*Significantly different from control value ($p \leq 0.05$).

TABLE 5. Residue Analyses in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Residue Levels (ppm); range or mean ±SD					
	Blood		Urine		Thyroid	
	ETU	EBDC	ETU	EBDC	ETU	EBDC[a]
Males						
0	<0.07 to 0.14	<0.80[b]	0.10±0.07	<0.04 to 0.2	<5.9 to 13	--
20	<0.12 to 0.16	<0.80	0.32±0.22	0.12±0.07	<5.6 to 8.8	--
80	0.18±0.15	<0.80	8.9 ±5.9	0.66±0.5	5.1±2.0	--
320	0.14±0.04	0.86±0.13	13.0 ±9.6	0.53±0.32	7.7±2.5	--
Females						
0	<0.07 to 0.22	<0.80	0.11±0.07	<0.01 to 0.6	<10 to 14	--
20	<0.10 to 0.14	<0.80 to 1.6	0.17±0.68	0.29±0.13	<5.9 to 13	--
80	0.17±0.10	<0.80	16.0 ±8.3	1.3 ±0.83	11.0±5.1	--
320	0.45±0.22	0.91±0.5	69.0 ±66	3.1 ±2.2	28.0±21	--

[a]Not analyzed due to limited sample size.

[b]Values that include symbol for less than (<) indicate the concentration was below the limits of detection.

H. Absolute and Relative Organ Weights:

1. 4 Weeks of Exposure: The authors reported no effects on thyroid or lung weights of males or females in any group after 4 weeks of exposure.

2. 13 Weeks of Exposure: Absolute kidneys and heart weights were significantly reduced ($p \leq 0.05$) in males exposed to 320 mg/m^3 after 13 weeks of exposure. The authors concluded that these reductions resulted from the reduced terminal body weights, and were secondary effects of exposure to mancozeb. No other absolute or relative organ weight effects were observed in either sex in any other group. Table 6 presents mean absolute and relative lung, thyroid, kidney, and heart weights for males and females after 13 weeks of exposure.

I. Ophthalmology: After 12 weeks of exposure to mancozeb, no exposure-related effects were observed in the eyes of any animal in any group. Bilateral retinal degeneration was observed in all of the animals whose cages were in the topmost position on the rack, and was attributed to an excessive amount of room light reaching these cages. One occurrence of ureitis and scattered occurrences of focal retinopathy were noted but were not considered exposure related by the authors.

J. Histopathology:

1. 4 Weeks of Exposure: Several gross and microscopic changes were seen in the tissues examined from the 4-week necropsy. These were scattered among the dosage and control groups, and the authors did not consider any of these changes to be exposure related.

2. 13 Weeks of Exposure: No exposure-related lesions were observed in males or females exposed to 20 mg/m^3 after 13 weeks of exposure to mancozeb. Males and females exposed to 80 or 320 mg/m^3 exhibited yellow-brown granular pigment in the lumen of the cortical tubules of the kidney. The authors considered this to be the result of the elimination of a pigmented metabolite which was considered to be produced as a consequence of exposure, but not toxicologically significant because there were no histopathologic changes seen in the kidney in animals of either group.

   The occurrence of mild hyperplasia of the follicular epithelium in the thyroid glands of three females exposed to 320 mg/m^3 was considered to be related to exposure. No exposure-related lesions were seen in the thyroid glands of male rats.

TABLE 6. Selected Organ Weight Data for Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Group Mean Value (± SD) at 13 Weeks							
	Lung		Kidneys		Heart		Thyroid	
	Absolute (g)	Rel. to BW (x 1000)	Absolute (g)	Rel. to BW (x 1000)	Absolute (g)	Rel. to BW (x 1000)	Absolute (mg)	Rel. to BW (x 1000)
Males								
0	2.55±0.29	6.01±0.61	3.54±0.29	8.35±0.69	1.64±0.15	3.87±0.31	28.9±4.1	0.068±0.011
20	2.48±0.29	5.89±0.56	3.25±0.43	7.73±0.83	1.53±0.17	3.64±0.29	26.5±2.4	0.063±0.007
80	2.67±0.79	6.25±1.86	3.48±0.29	8.15±0.52	1.61±0.12	3.78±0.27	26.0±5.1	0.061±0.012
320	2.44±0.31	6.08±0.64	3.14*±0.32	7.84±0.71	1.48*±0.13	3.70±0.20	26.9±3.3	0.067±0.009
Females								
0	1.86±0.16	8.53±0.89	2.10±0.13	9.65±0.84	1.00±0.07	4.59±0.51	20.5±3.5	0.095±0.018
20	1.99±0.35	8.38±1.85	2.20±0.31	9.17±0.93	1.07±0.10	4.46±0.28	20.6±4.5	0.086±0.018
80	1.84±0.17	8.20±0.64	2.15±0.30	9.55±1.20	1.00±0.09	4.45±0.33	22.0±2.2	0.098±0.008
320	2.09±0.38	9.08±1.07	2.32±0.70	10.84±2.48	1.06±0.14	4.64±0.62	22.1±2.4	0.097±0.011

*Significantly different from control value ($p \leq 0.05$).

Several histopathologic lesions were noted in the respiratory tract, but these were considered to be spontaneous and not related to exposure to mancozeb. Table 7 presents the incidence of findings in the respiratory tract, thyroid, and kidney.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Four groups of 38 male and 38 female rats were exposed to target dust aerosol concentrations of 0, 20, 80, and 320 mg/m^3 for 13 weeks. The actual respirable concentrations for the 13 weeks were 0, 8, 36, and 144 mg/m^3, respectively.

After 4 weeks of exposure, significant ($p < 0.05$) reductions in body weight and body weight gain were noted for male rats exposed to the target concentration of 320 mg/m^3. No other effects were considered to be related to exposure by the authors.

After 13 weeks of exposure, significant reductions were found in body weight and body weight gain in males as well as reductions in T4 levels in females exposed to the target concentrations of 320 mg/m^3. In addition, microscopic examination of the thyroid glands in these females revealed hyperplasia of the follicular epithelium. No other effects were considered to be related to exposure to mancozeb.

There was a yellow-brown granular pigment in the convoluted tubules of male and female rats exposed to 80 or 320 mg/m^3. The authors did not consider this pigment a manifestation of toxicity but likely to be the accumulation of the metabolite, ethylenebisisothiocyanate sulfate.

Based on these results, the NOAEL for mancozeb in rats was considered to be 36 mg/m^3 and the LOAEL was consider to be 144 mg/m^3 based on respirable concentration data.

B. Signed quality assurance statements were dated February 27, 1986 for toxicity and January 13, 1985 for the residue report (Note: this is probably an error in year).

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Considerable variation in chamber concentrations was noted, especially early in this study. The ranges in daily mean chamber concentrations were narrower after the first 4 weeks. The characterization of the dust aerosol was adequate, and it is appropriate to base the toxicological findings on the respirable concentrations of 0, 8, 36, and 144 mg/m^3 for the target concentrations of 0, 20, 80, and 320 mg/m^3, respectively, as reflected in the following assessment.

TABLE 7 Incidence of Selected Histopathologic Lesions Found in Rats Exposed to Maneb for 13 Weeks

Organ/Finding	Target Exposure Concentration (mg/m³)							
	Males				Females			
	0	20	80	320	0	20	80	320
Kidney								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
yellow-brown pigment, cortical tubules	0	0	5	8	0	0	10	9
Thyroid								
No. examined	(10)	(10)	(10)	(11)	(10)	(10)	(10)	(10)
-hyperplasia, follicular epithelium	0	0	0	0	0	0	0	3
Lung								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
-multifocal interstitial inflammation	3	3	1	4	9	1	*	8
-foci of alveolar macrophages	5	0	0	3	2	0	*	4
--focal/multifocal hemorrhage	0	0	0	1	0	0	*	0
--diffuse acute congestion	0	1	0	1	0	1	1	1
Nasal Turbinates								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--congestion	0	0	0	0	0	0	1	1
--multifocal mononuclear cellular infiltration	2	2	3	4	0	1	0	2
--hemorrhage	0	0	0	0	0	0	1	1
Trachea								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--focal tracheitis	1	0	1	3	0	2	2	3

The body weight data revealed an exposure-related, significant reduction in body weight and weight gain for male rats exposed to 144 mg/m^3. A significant increase in body weight gain in females exposed to 8 mg/m^3 was also noted; however, these females were slightly smaller at study initiation, and the effect was not seen at higher exposure levels; this weight gain is therefore not considered related to exposure.

The hematology data reflect the general good health of these rats with low white cell counts and no unusual data among the red cell indices. The significant increase in mean corpuscular volume and decrease in mean corpuscular hemoglobin concentration are within the normal range of values for rats and were not considered to be related to exposure to mancozeb.

Triglyceride levels in males exposed to 144 mg/m^3 and inorganic phosphorus levels in females exposed to 36 mg/m^3 were significantly reduced when compared to controls. These changes were within the normal range of values for rats and were not considered to be related to exposure to mancozeb.

The significant reduction in serum T4 levels in females exposed to 144 mg/m^3 noted at week 13 was considered to be related to exposure to mancozeb.

Residue analyses in blood, urine, and thyroids revealed increasing concentrations of ethylenethiourea and ethylenebisdithiocarbamate with increasing exposure concentration. These data support the hypothesis that mancozeb is metabolized to ethylenethiourea and that the thyroid is a target organ for mancozeb toxicity.

The absolute weights of kidneys and heart were significantly reduced in males exposed to 144 mg/m^3. The authors concluded that these differences were due to reduced body weight in these males. However, we calculated organ-to-brain weight ratios and found that the relative kidneys and heart weights were reduced, although not significantly for males exposed to 144 mg/m^3.

No remarkable findings were noted during the ophthalmological examinations.

Microscopic examination of tissues taken at termination (13 weeks) necropsy revealed the presence of yellow-brown, granular pigment in males and females exposed to 36 mg/m^3. The pigment was not present at the 4-week interim sacrifice and, thus, may represent possible progressive lesions in the kidney. In addition, mild hyperplasia of the follicular epithelium of the thyroid occurred in three females exposed to 144 mg/m^3. These changes may be due at least in part to a compensatory reaction to hypothyroidism. Since hyperplasia was not present after the initial 4 weeks of exposure, it is possible that these changes indicate progressive

lesions in female rats. As expected for dust aerosol exposures, there were minor histologic changes in the nasal turbinates and trachea as well as congestion in the lungs of rats exposed to mancozeb. These changes may indicate progressive lung disease leading to the formation of granulomas in the lung and bronchial lymph nodes. Based on these histologic findings, the thyroid, kidneys, and respiratory tract are target organs in rats exposed to dust aerosols of mancozeb.

B. The study was conducted in an acceptable manner and appropriate quality assurance inspections were reported.

C. The effects on body weight in males and on the thyroid in females are similar to effects seen in other studies of mancozeb. The findings of the yellow-brown granular pigment in the kidneys, accompanied by a decrease in kidney weight, has not been reported previously. This may mean that the metabolic fate from inhaled mancozeb is at least slightly different from the fate of ingested mancozeb. The fact that the pigment is granular may indicate a serious kidney problem in rats exposed beyond 13 weeks. These inclusions were not present at the 4-week interim sacrifice while the incidence was high in animals exposed to 36 or 144 mg/m^3. These inclusions may represent a form of kidney urolithiasis as seen with gout, cystinurea, or hyperoxaluria. Stones can form from oxalatea cysteine, uric acid, calcium carbonate, or calcium phosphate. Uric acid is particularly sensitive to pH with stones forming below pH 5.5. The exact nature of mancozeb and/or its metabolites in kidney function is not known; however, these renal inclusions cannot be ignored. An ongoing recovery study will reveal whether or not the pigment is cleared from the kidneys over time. The changes in the respiratory tract are not unexpected for dust aerosol exposures. These types of lesions can progress to the formation of granulomas in the lung and microgranulomas in the bronchial lymph nodes. The ongoing recovery study will give some indications of whether or not these are progressive lesions of the respiratory tract.

Based on these results, there are definite effects of exposure to a respirable concentration of 144 mg/m^3 mancozeb; for males in this study it was body weight and for females effects were on thyroid function and were verified by histology on the thyroid. The seriousness of the renal inclusions (granular pigment) cannot be assessed. The inclusions were not present at 4 weeks, while the incidence was high in the 36 and 144 mg/m^3 groups at study termination. Thus, we cannot ignore inclusions and based on these data, the LOAEL for rats exposed to mancozeb is 36 mg/m^3 and the NOAEL is 8 mg/m^3.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol.

Mancozeb TOX Review 005425

Page____ is not included in this copy.

Pages 99 through 146 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA: 68-02-4225
DYNAMAC No. 205-B
September 12, 1986

005425

DATA EVALUATION RECORD

MANCOZEB

Metabolism in Rats

STUDY IDENTIFICATION: DiDonato, L. J. and Longacre, S. L. Mancozeb pharmacokinetic study in rat (unpublished study No. B5R-123); Longacre, S. L. Summary of ETU and EBDC analyses in plasma, liver, and thyroid after mancozeb administration (unpublished study No. B5R-123); Nelson, S. S. Metabolism of C-14 mancozeb in rat (unpublished study No. 31H-86-02).

Prepared by Rohm and Haas Company, Philadelphia, PA; dated May 21/22, 1986. Accession Nos. 262834 and 262835.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

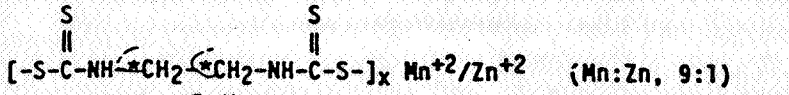
Signature: I Cecil Felkner

Date: 9-12-86

147

1. CHEMICAL: Mancozeb; ethylene-bis-dithiocarbamate, Zn^{+2}, Mn^{+2} coordination product; Dithane M-45 fungicide.

2. TEST MATERIAL: [^{14}C-ethylene]Mancozeb from lot Nos. 476.0108 and 476.0201 with specific activities of 11.60 and 11.54 mCi/g, respectively, was used. The following is the chemical structure of mancozeb:



* location of C-14

Also, technical mancozeb from lot No. 43339 containing 84 percent active ingredient was used.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: DiDonato, L. J. and Longacre, S. L. Mancozeb pharmacokinetic study in rats (unpublished study No. 85R-123); Longacre, S. L. Summary of ETU and EBDC analyses in plasma, liver, and thyroid after mancozeb administration (unpublished study No. 85R-123); Nelson, S. S. Metabolism of C-14 mancozeb in rat (unpublished study No. 31H-86-02).

Prepared by Rohm and Haas Company, Philadelphia, PA; dated May 21/22, 1986. Accession Nos. 262834 and 262835.

5. REVIEWED BY:

Charles Rothwell, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: _____
Date: _____

Nicolas P. Hajjar, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: _____
Date: _____

6. APPROVED BY:

William L. McLellan, Ph.D.
Metabolism
Technical Quality Control
Dynamac Corporation

Signature: _____
Date: _____

Irving Mauer, Ph.D.
EPA Reviewer

Signature: [signature]
Date: 04-09-86

ADD:- Jane E. Harris, Ph.D.
Section Head.

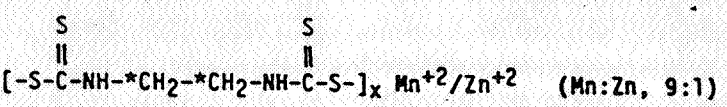
Signature: [signature]
Date: 9/9/86

143

1. CHEMICAL: Mancozeb; ethylene-bis-dithiocarbamate, Zn^{+2}, Mn^{+2} coordination product; Dithane M-45 fungicide.

005425

2. TEST MATERIAL: [^{14}C-ethylene]Mancozeb from lot Nos. 476.0108 and 476.0201 with specific activities of 11.60 and 11.54 mCi/g, respectively, was used. The following is the chemical structure and position of the labeled carbons (*) of [^{14}C]mancozeb:



$$[-S-\overset{\overset{S}{\|}}{C}-NH-{}^{*}CH_2-{}^{*}CH_2-NH-\overset{\overset{S}{\|}}{C}-S-]_x \; Mn^{+2}/Zn^{+2} \quad (Mn:Zn,\ 9:1)$$

Also, unlabeled technical mancozeb from lot No. 43339 containing 84 percent active ingredient was used.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: DiDonato, L. J. and Longacre, S. L. Mancozeb pharmacokinetic study in rats (unpublished study No. 85R-123); Longacre, S. L. Summary of ETU and EBDC analyses in plasma, liver, and thyroid after mancozeb administration (unpublished study No. 85R-123); Nelson, S. S. Metabolism of C-14 mancozeb in rat (unpublished study No. 31H-86-02).

Prepared by Rohm and Haas Company, Philadelphia, PA; dated May 21/22, 1986. Accession Nos. 262834 and 262835.

5. REVIEWED BY:

Charles Rothwell, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Charles Rothwell
Date: 9-12-86

Nicolas P. Hajjar, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: 9-12-86

6. APPROVED BY:

William L. McLellan, Ph.D.
Metabolism
Technical Quality Control
Dynamac Corporation

Signature: William L McLellan
Date: 9-12-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irving Mauer
Date: 09-17-86

7. CONCLUSIONS:

A. Male and female rats given single oral doses of [^{14}C]mancozeb at 1.5 or 100 mg/kg absorbed approximately half of the dose. Absorption was rapid; peak plasma concentrations of [^{14}C] were reached within 3 and 6 hours after dosing at 1.5 and 100 mg/kg, respectively. Elimination of [^{14}C] from the plasma was biphasic with half-lives for the rapid and slow phases ranging from 3.9 to 6.1 and 23.1 to 38.5 hours, respectively. Most of the oral dose was excreted within 24 hours of administration. At 96 hours postdosing, about half the dose was eliminated in the urine and half in the feces; less than 2 and 4 percent were found in expired air and tissues, respectively. Most of the [^{14}C] eliminated in the feces represented unabsorbed material since only 2.0 to 8.8 percent of an oral dose was excreted in the 0- to 24-hour bile. Thyroid contained the highest [^{14}C] residue levels. Peak thyroid concentrations were not proportional to dose and were disproportionately less than the respective peak blood [^{14}C] concentrations after a 100-mg/kg dose than after a 1.5-mg/kg dose. The disposition or excretion of [^{14}C]mancozeb in rats fed diets containing technical mancozeb at 15 ppm active ingredient for 14 days prior to being dosed with [^{14}C]mancozeb at 1.5 mg/kg was not significantly different from that observed in the above-mentioned groups.

Metabolite analysis revealed that mancozeb is extensively metabolized and/or degraded by the rat. Ethylenethiourea (ETU) was a major degradate/metabolite found in urine, bile, and feces. ETU was also measured in plasma, liver, and thyroid and was found to have an elimination half-life of 3.9 to 4.7 hours in those tissues. Ethylene-bis-dithiocarbamate (EBDC), the major component of mancozeb, was detected in liver, feces, and bile, but not in thyroid. Other degradates/metabolites identified in feces, urine, and/or bile include ethylenebis-(isothiocyanate) sulfide (EBIS), ethyleneurea (EU), N-acetyl-ethylenediamine (N-AcEDA), and ethylenediamine (EDA). Degradates/metabolites tentatively identified were glycine (Gly), N-acetylglycine (N-AcGly), and N-formylethylenediamine (N-ForEDA). Five unknowns were also found, one of which was proposed to be ethylenethiuram disulfide (ETD). Structures and the proposed degradation/metabolic pathway for mancozeb are presented in Figure 1. The quantities of these metabolites arising from in vivo metabolism/degradation of mancozeb cannot be determined precisely because of the presence of 8-12 percent [^{14}C] impurities in the test material.

B. This metabolism study is acceptable.

Item 8-10--see footnote 1.

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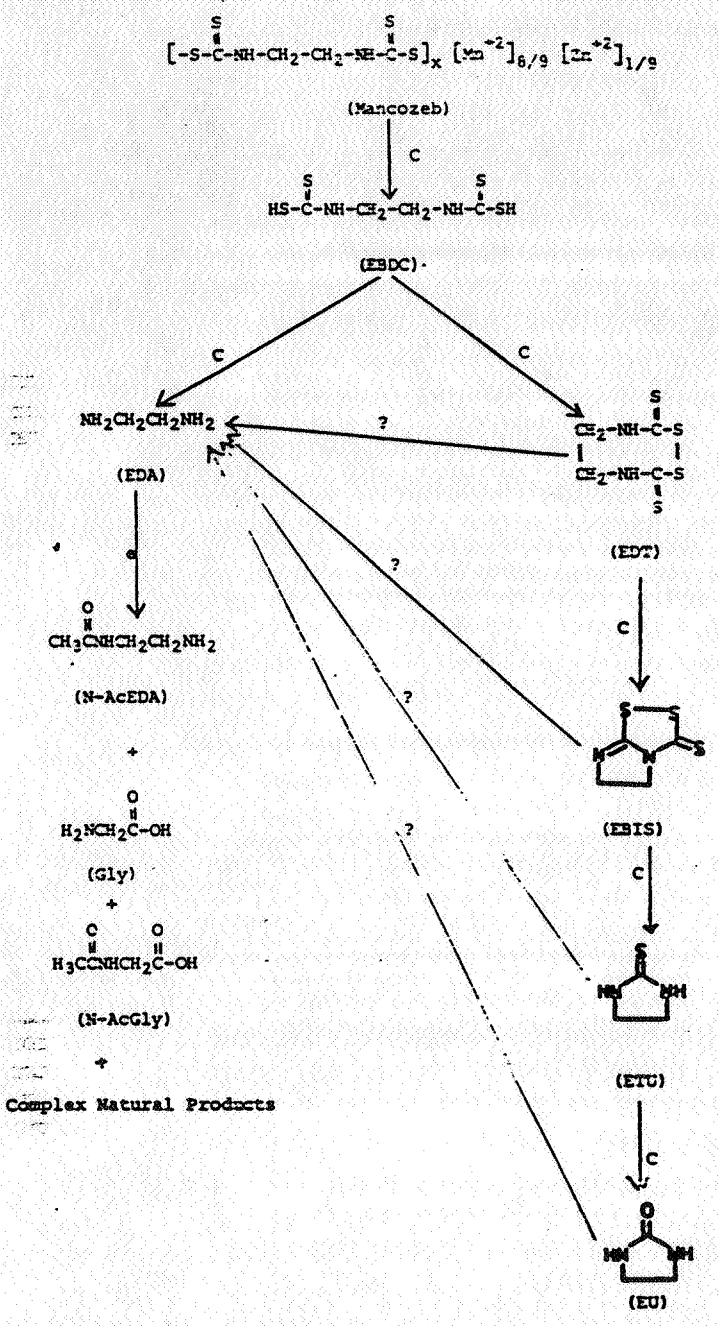
[1]Only items appropriate to this DER have been included.



c: Known chemical conversions
e: Enzymatic conversions
?: Unknown whether these reactions could occur either chemically or enzymatically.

Figure 1. Proposed Degradation/Metabolic Pathway for Mancozeb

II. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test materials were unlabeled technical mancozeb (84 percent active ingredient) and [^{14}C-ethylene]mancozeb with a specific activity of approximately 11.55 mCi/g. Analysis of the [^{14}C]mancozeb by high pressure liquid chromatography (HPLC) indicated a radiochemical purity of 88 to 92 percent. The number and identities of the radiolabeled impurities were not reported.

2. The test animals were 3- to 9-week-old male and female Sprague-Dawley CD rats supplied by Charles River Laboratories. At the time of dosing, the animals weighed between 75 and 300 g.

3. The radiolabeled test material was suspended in 0.5 percent methylcellulose and was administered by gavage. The [^{14}C]-mancozeb was not diluted with nonradiolabeled mancozeb except to dose two rats at 100 mg/kg in a range-finding study; the specific activity of the mancozeb given those animals was 4.99 mCi/g.

4. A preliminary range-finding study was performed with groups of two male rats administered single oral doses of [^{14}C]mancozeb at 1.5, 100, or 300 mg/kg. After dosing, one rat each dosed with 1.5 and 100 mg/kg and both rats dosed with 300 mg/kg were placed in individual metabolism cages for the collection of urine and feces. Expired air was also collected for rats receiving 1.5 and 100 mg/kg. Collection times were 6, 24, 48, 72, and 96 hours. The animals were sacrificed at 96 hours, and plasma, whole blood, thyroid, liver, adipose tissue, kidney, lung, heart, bone marrow, testes, muscle, and spleen were taken for radioassay. Blood samples were collected from the other two rats dosed with 1.5 and 100 mg/kg at 1, 3, 6, 24, 48, and 72 hours after dosing.

5. For the main study, 17 male and 17 female rats per group were administered single oral doses of [^{14}C]mancozeb at 1.5 or 100 mg/kg. Three rats/sex/group were sacrificed at 1, 6, 24, and 48 hours postdosing, and their plasma, whole blood, liver, and thyroids were taken for radioassay and metabolite analysis. Additionally, blood samples were taken at 0.5 and 3 hours from the rats sacrificed at 1 and 6 hours, respectively. Five rats/sex/group were placed in individual metabolism cages immediately after dosing, and their urine and feces were collected at 6, 24, 48, 72, and 96 hours postdosing. The remaining animals were sacrificed at 96 hours, and their plasma, whole blood, thyroids, liver, adipose tissue, kidneys, lung, heart, bone marrow, gonads, muscle, spleen, and brain were collected for radioassay and metabolite analysis.

An additional 10 male and 10 female rats were placed on diets of Purina Rodent Chow containing unlabeled technical mancozeb at 15 ppm (active ingredient). After 14 days on the mancozeb diet, five rats/sex/group were given single oral doses of [^{14}C]mancozeb at 1.5. After dosing, the animals were housed in individual metabolism cages and urine and feces collected as described above. The animals were sacrificed 96 hours postdosing and tissues were collected as described above.

Finally, two groups of three male and three female rats with cannulated bile ducts were administered single oral doses of [^{14}C]mancozeb at 1.5 or 100 mg/kg. Bile was collected during 0-6 and 6-24 hours postadministration for radioassay and metabolite analysis. No other samples were taken from these animals.

6. Samples of urine and feces were collected over dry ice as much as possible and stored frozen until analyzed. Expired air collected in the range-finding study was trapped in 5 percent sodium hydroxide. Aliquots of blood were centrifuged to collect plasma for radioassay. Liver, thyroid, and plasma samples to be analyzed for ETU and EBDC content were kept frozen until analysis. Aliquots of urine, blood, bile, and other liquid samples were radioassayed by liquid scintillation counting (LSC). Solid samples were combusted and the combustion products radioassayed by LSC. The percent of dose in bone marrow, adipose tissue, and muscle was calculated assuming these tissues represented 0.4, 7.0, and 45.5 percent of body weight, respectively. The percent of dose in whole blood was calculated assuming whole blood represented 6.5 percent of body weight.

7. Plasma, liver, and thyroid samples remaining after aliquots were taken for [^{14}C] quantitation were pooled for sex and dose group. The pooled samples were analyzed for ETU and EBDC content using a modification[2] of a previously published HPLC technique.[3] Further details on the modified methodology used were not reported.

8. Following radioassay, the remaining samples of urine and feces collected 6, 24, 48, 72, and 96 hours after dosing were pooled according to sex and dose group so that the pooled samples contained over 90 percent of the excreted radioactivity. These pooled samples were used for metabolite identification. Similarly, the 0- to 6-hour and 6- to 24-hour bile samples were pooled for identification of metabolites.

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[2] Rohm and Haas. Technical Report No. TR36F-82-15, March 4. 1982, Ag. Prod. Environ. Sciences.

[3] Haines, L.D. and Adler, I.L. Gas chromatographic determination of ethylenethiourea residues. J.A.O.A.C. 56:333-337 (1973).

9. Radiolabeled metabolites in urine, feces, and bile were separated by thin-layer chromatography (TLC) and/or HPLC. Radioactive spots on developed TLC plates were located by autoradiography and quantitated by LSC. Metabolite identification was done by cochromatography with known standards. Details on the methodology used for isolation, identification, and quantitation of [^{14}C]mancozeb metabolites/degradates are included in Appendix A of this review.

B. Protocol: A protocol for the study design was included with the report (see Appendix B of this review).

12. REPORTED RESULTS:

A. Actual Doses: The actual doses of [^{14}C]mancozeb orally administered to each animal were presented. An examination of the data indicated that each rat received 95 to 105 percent of the nominal concentration. Analysis of the unlabeled mancozeb-treated feed showed that the average mancozeb concentration to be 99 percent of nominal (15 ppm) and the ETU content was approximately 0.4 ppm.

B. Range-Finding Study: The total recovery of [^{14}C] from animals in the range-finding study 96 hours after dosing ranged from 90.4 to 121.7 percent of the dose. For the rats dosed at 1.5 or 100 mg/kg, approximately 25 percent of the dose was excreted in urine collected at 0-96 hours and 77-89 percent in feces collected at 0-96 hours. Expired air contained only 0.3-1.3 percent of the dosed [^{14}C]. The [^{14}C] excretion pattern for the rats dosed at 300 mg/kg was quite different: 41.6 and 44.0 percent of the dose being excreted into the feces and urine after 96 hours, respectively.

Plasma and whole blood levels of [^{14}C] peaked within 6 hours of dosing, accounting for 0.475 and 15.45 ppm in male rats dosed at 1.5 and 100 mg/kg, respectively, in the range-finding study. The elimination of radioactivity was biphasic, and [^{14}C] concentrations in plasma and whole blood were essentially similar at each time point for each dose. Of the tissues analyzed 96 hours post-administration, thyroid contained the highest [^{14}C] levels at all three dose levels, followed by liver, kidney, bone marrow, and lung, accounting for 107, 40, 37, 22, and 18 ppm, respectively.

C. Main Study: The average total recoveries of radioactivity per group at 0-96 hours after dosing with [^{14}C]mancozeb ranged from 92.0 to 124.4 percent of dose. The mean excretion of radioactivity in urine and feces following oral administration of [^{14}C]mancozeb is presented in Table 1. Most (73.95 to 94.14 percent) of the administered dose was recovered in the excreta within 24 hours of dosing; 87.57 to 119.7 percent of the dose (95 to 98 percent of the recovered [^{14}C]) was eliminated in the excreta by 96 hours. The excreted radioactivity was essentially

TABLE 1. Mean Excretion of Radioactivity in Urine and Feces Following Oral Administration of [^{14}C]Mancozeb at 1.5 or 100 mg/kg to Male (M) and Female (F) Rats

		Mean recovery of [^{14}C] as percent of administered dose[a]					
		1.5-mg/kg dose		100-mg/kg dose		Multiple doses[b]	
Time (hours)		M	F	M	F	M	F
Urine	0-6	20.50	23.79	13.59	15.08	10.84	10.32
	6-24	23.40	20.12	27.89	30.07	37.71	36.02
	24-48	4.00	3.80	7.07	6.69	5.42	4.03
	48-72	0.67	1.10	1.57	1.78	1.07	0.89
	72-96	0.29	0.72	0.87	0.85	0.62	0.42
	Total	48.86	49.53	50.99	54.47	55.66	51.68
Feces	0-6	1.02	10.81	13.05	1.54	0.12	1.68
	6-24	49.22	31.65	37.58	44.11	30.43	25.93
	24-48	4.59	3.29	7.00	15.62	9.57	7.43
	48-72	0.37	0.65	0.66	3.82	0.75	0.64
	72-96	0.12	0.21	0.18	0.12	0.21	0.21
	Total	55.32	46.61	58.47	65.21	41.08	35.89

[a] N = 5.

[b] These animals were given single oral doses of [^{14}C]mancozeb at 1.5 mg/kg after 14 days on diets containing unlabeled technical mancozeb at 15 ppm active ingredient.

evenly distributed between the urine and feces for all three dose groups.

Individual animal data on the biliary excretion of [^{14}C] after dosing with [^{14}C]mancozeb are presented in Table 2. Approximately 6.3 to 8.8 and 2.0 to 3.8 percent of the dose were excreted by rats within 24 hours postdosing at 1.5 and 100 mg/kg, respectively.

Concentrations of [^{14}C] in the plasma of male and female rats administered single oral doses of [^{14}C]mancozeb at 1.5 or 100 mg/kg are presented in Figures 2 and 3, respectively. The [^{14}C] plasma concentrations for males and females were very similar for each dose group. Radioactivity was rapidly absorbed into the plasma with half-lives (t 1/2 absorption) of 0.7 to 1.0 hour for the low-dose group and 1.7 hours for the high-dose group. Peak concentrations of 0.32 to 0.33 ppm were achieved within 3 hours for the low-dose group. Peak concentrations for the high-dose group of 18.1 to 18.7 ppm were reached within 6 hours after dosing. The elimination of radioactivity from plasma was biphasic; the half-lives for the rapid elimination were 3.9 and 5.7 hours for males receiving the low and high doses, respectively, and 4.6 and 6.1 hours for females receiving the low and high doses, respectively. The rapid phase of elimination in the high-dose group was much less pronounced than in the low-dose group. The slow elimination half-lives for both sexes were about 36.5 and 25 hours in animals receiving the low and high doses, respectively.

[^{14}C] levels in whole blood were similar to the corresponding plasma concentrations for each dose group; however, the slow phase of elimination for the low-dose group was longer in blood (t 1/2 = 69.3 to 138.6 hours) compared to plasma (t 1/2 = 34.7 to 38.5 hours).

Concentrations of [^{14}C] in liver were very similar between males and females at each time point after dosing with [^{14}C]mancozeb (Figures 4 and 5). Peak levels were reached within 6 hours (1 hour for the low-dose females) of dosing and were 1.6- to 1.8-fold (0.71-0.81 ppm) and 5.6- to 6.3-fold (100.3-113.9 ppm) higher than the corresponding peak [^{14}C] whole blood concentrations after 1.5- and 100-mg/kg doses, respectively. Elimination kinetics were biphasic; the half-lives for the rapid and slow elimination phases were 6.97 to 8.70 and 31.99 to 37.70 hours, respectively.

Figures 6 and 7 show the concentrations of [^{14}C] in thyroid at various times after administration of [^{14}C]mancozeb at 1.5 or 100 mg/kg to male and female rats, respectively. The individual animal values varied by as much as 30-fold within a group. Peak levels were reached within 6 and 24 hours in animals receiving the low and high doses, respectively; [^{14}C] levels decreased

TABLE 2. Biliary Excretion of Radioactivity Following Administration of [^{14}C]Mancozeb at 1.5 or 100 mg/kg to Rats

Sex	Dose (mg/kg)	0-6 Hours Volume (mL)	0-6 Hours Percent of dose	6-24 Hours Volume (mL)	6-24 Hours Percent of dose	Total Volume (mL)	Total Percent of dose
M	1.5	5.0	7.21	9.0	1.54	14.0	8.75
M[a]	1.5	3.0	2.93	3.0	1.62	6.0	4.57
mean						14.0	8.75
F	1.5	3.4	3.20	8.8	2.97	12.2	6.17
F	1.5	2.0	3.04	8.6	3.42	10.6	6.47
F[a]	1.5	1.8	3.43	0.5	0.55	2.3	3.98
mean						11.4	6.32
M	100	3.5	1.31	11.4	2.72	14.9	4.03
M	100	3.7	1.21	11.0	2.82	14.7	4.03
M	100	3.9	1.38	11.6	1.82	15.5	3.20
mean						15.0	3.75
F	100	4.8	0.05	13.9	0.29	18.7	0.34
F	100	3.9	0.04	7.7	0.10	11.6	0.14
F	100	3.4	1.28	10.4	2.35	13.8	3.63
mean						16.3	1.98

[a]Data from these animals were not used by the study authors to calculate the means due to "encountered technical difficulties."

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between 24 and 48 hours, and then increased or remained constant between 48 and 96 hours. The peak [^{14}C] levels in thyroid were 42.1- to 45-fold (18.8-20.8 ppm) and 5.6- to 15.7-fold (100.7-282.9 ppm) higher than the corresponding peak [^{14}C] levels in whole blood after 1.5 and 100 mg/kg doses, respectively.

The [^{14}C] tissue distribution 96 hours after dosing with [^{14}C]mancozeb are presented in Table 3. For each group and tissue, residue levels were comparable between males and females, except for adipose tissue and gonads, in which the [^{14}C] concentrations in tissues from females were 2- to 11-fold higher than the respective concentrations found in the tissues of male rats. The thyroid contained the highest residue levels for each group; it was 9.8- to 18.7-fold higher than the next highest tissue concentrations of [^{14}C] (liver, kidney, and spleen). However, the thyroid contained only 0.03 (1.5-mg/kg dosed males) to 0.10 percent of the dose (100-mg/kg dosed females) 96 hours after dosing. Tissue concentrations of [^{14}C] residues in rats fed diets containing unlabeled technical mancozeb at 15 ppm active ingredient for 2 weeks prior to dosing with [^{14}C]mancozeb at 1.5 mg/kg were comparable to those in animals receiving only single doses of [^{14}C]mancozeb at 1.5 mg/kg.

The average [^{14}C] residue levels per group remaining in the tissues of rats 96 hours after dosing were only 1.58 to 3.69 percent of the dose.

D. ETU and EBDC Levels in Plasma, Liver, and Thyroid: Concentrations of ETU and total [^{14}C] residues in plasma, liver, and thyroid of rats dosed with [^{14}C]mancozeb are presented in Table 4. ETU concentrations in plasma and liver of rats 6 hours after dosing with [^{14}C]mancozeb at 1.5 mg/kg were 5.4- to 7.0-fold and 11.7- to 12.6-fold less than the corresponding plasma and liver [^{14}C] levels, respectively. ETU was not detected in the pooled thyroids of these low dose animals.

For animals administered single oral doses of [^{14}C]mancozeb at 100 mg/kg, the peak plasma levels of ETU were reached 6 hours postadministration and were about 6- and 13-fold less than the corresponding plasma [^{14}C] levels for males and females, respectively. ETU was rapidly eliminated from the plasma of both male and female rats ($t_{1/2}$ for elimination = 3.9 to 4.7 hours); ETU had decreased below detectable levels (0.017 ppm) within 48 hours after dosing. The area under the plasma ETU concentration vs. time curve (AUC) was 6.4 and 3.1 percent of the AUC for the plasma [^{14}C] concentrations vs. time curve for males and females, respectively.

ETU concentrations in the liver of high-dose rats were 95- to 107-fold less than the corresponding liver [^{14}C] concentrations 6 hours after dosing. ETU was rapidly eliminated from the liver, decreasing below detectable levels (0.014 ppm) within 48 hours after dosing.

TABLE 3. Mean Concentrations of Radioactivity in Various Tissues 96 Hours After Administration of a Single Oral Dose of [^{14}C]Mancozeb in Male (M) and Female (F) Rats

Tissue	Mean tissue concentration of [^{14}C]mancozeb residues (ppm)[a]					
	1.5-mg/kg dose		100-mg/kg dose		Multiple doses[b]	
	M	F	M	F	M	F
Blood	0.104	0.089	1.34	1.93	0.084	0.095
Liver	0.035	0.034	4.19	5.71	0.110	0.112
Thyroid	4.42	6.52	79.0	131.7	9.16	9.20
Spleen	0.017	0.029	1.49	2.62	0.060	0.078
Kidney	0.364	0.433	4.87	7.06	0.939	0.675
Heart	0.016	0.027	1.30	2.07	0.049	0.056
Lung	0.019	0.029	1.58	2.34	0.057	0.060
Brain	0.022	0.013	0.358	0.514	0.012	0.013
Bone marrow	0.375	0.421	1.84	2.75	0.241	0.347
Adipose tissue	0.030	0.119	1.01	2.94	0.045	0.091
Gonads	0.009	0.095	0.842	2.54	0.022	0.142
Muscle	0.011	0.017	1.45	1.73	0.038	0.040

[a] N = 5.

[b] Animals received a single oral dose of [^{14}C]mancozeb at 1.5 mg/kg after 2 weeks on a diet containing technical mancozeb at 15 ppm active ingredient.

TABLE 4. Concentrations of ETU and [^{14}C] in Plasma, Liver, and Thyroid of Rats Dosed with [^{14}C]Mancozeb

Dose Group	Sex	Time Postdosing (hours)	Concentration (ppm) in pooled samples[a]					
			Plasma		Liver		Thyroid	
			[^{14}C]	ETU	[^{14}C]	ETU	[^{14}C]	ETU
1.5 mg/kg	M	6	0.278	0.043	0.709	0.056	18.84	ND[b]
	F	6	0.256	0.047	0.538	0.046	20.75	ND
100 mg/kg	M	1	5.30	1.00	30.90	0.78	19.97	ND
	F	1	7.45	0.98	48.79	0.79	47.29	1.6
	M	6	18.14	2.86	113.9	1.20	64.27	0.62
	F	6	18.69	1.42	100.3	0.94	41.94	0.4
	M	24	6.16	0.12	19.04	0.15	100.7	1.3
	F	24	7.73	0.10	23.94	0.11	282.9	1.25
	M	48	3.91	ND	14.15	ND	28.33	ND
	F	48	3.92	ND	13.99	ND	21.96	ND
	M	96[c]	0.747	ND	4.19	ND	79.00	ND
	F	96[c]	1.18	ND	5.71	ND	131.7	ND
Multiple doses[d]	M	96[c]	0.024	ND	0.110	ND	9.158	ND
	F	96[c]	0.028	ND	0.112	ND	9.203	ND

[a] ETU was measured in pooled samples (n = 3).

[b] ND = not detected, the detection limits for ETU in plasma, liver, and thyroid were 0.017 ppm, 0.014 ppm, and 0.012 µg/sample, respectively.

[c] N = 5 for these pooled samples.

[d] Animals received a single oral dose of [^{14}C]mancozeb at 1.5 mg/kg after [illegible] containing technical mancozeb at 15 ppm active ingredient.

In male rats dosed at 100 mg/kg, ETU concentrations in the thyroids were 78- and 104-fold less than the corresponding thyroid [^{14}C] concentrations at 6 and 24 hours, respectively. In females, ETU concentrations in the thyroids were 30- to 226-fold less than the corresponding thyroid [^{14}C] concentrations at 1, 6, and 24 hours postadministration.

ETU was not detectable in the plasma, liver, or thyroid of rats at 96 hours after administration of a single oral dose of [^{14}C]mancozeb at 1.5 mg/kg, which had followed 2 weeks of dietary intake of 15 ppm of unlabeled technical mancozeb.

Concentrations of EBDC in the liver of rats 1, 6, and 24 hours after dosing with [^{14}C]mancozeb at 100 mg/kg were 0.26, 0.61, and 0.29 ppm for males, respectively, and 0.32, 0.35, and 0.25 ppm for females, respectively. EBDC residues were not detected (<0.22 ppm) in the livers of male and female high-dose rats sacrificed 48 or 96 hours after dosing. Furthermore, EBDC was not detected in the livers of rats receiving the low dose (1.5 mg/kg) or the multiple doses 96 hours postadministration.

E. Metabolite Isolation and Identification: Extraction of the pooled fecal samples with EDTA/ethanol removed 33 to 57 percent of the radioactivity. A second extraction with ethanol/water removed another 20 to 43 percent of the radioactivity. The range of radioactivity remaining in the feces was 9-41 percent. The extracts were analyzed by TLC and the resulting metabolic profile is presented in Table 5 (see Figure 1 for structures). Both extracts of the 100-mg/kg group were analyzed whereas only the EDTA/ethanol extracts of the low-dose and multiple-dose groups were analyzed because of the small amount of [^{14}C] in the ethanol/water extracts. The amount of EBDC as determined by HPLC remained fairly constant, accounting for 6.9 to 9.9 percent of the total fecal [^{14}C] in the low-dose group, 6.8 to 12.3 percent in the high-dose group, and 7.6 to 11.0 percent in the multiple-dose group. However, when EBDC was quantitated by the CS$_2$ method [illegible] fecal [^{14}C] was derived [illegible] Although the fecal metabolite profiles were similar for both sexes, the percentage of ETU was greater in the high-dose group (8.3-14.2 percent) than in the low-dose group (2.1-4.1 percent) and multiple-dose group (2.4-5.2 percent).

The metabolite/degradate profile of urine from rats dosed with [^{14}C]mancozeb is presented in Table 6. The major metabolite/degradate was ETU, comprising 30.8 to 42.7 percent of the radioactivity in the urine. Whereas ETU appeared to be higher in the 100-mg/kg group, it probably is not significantly higher. Most of the labeled compounds found in the feces were also found in the urine.

TABLE 5. Metabolite Profile in Fecal Samples from Male (M) and Female (F) Rats Dosed with [^{14}C]Mancozeb

Metabolite/ Degradate	Percent of [^{14}C] in Feces[a]					
	1.5-mg/kg dose		100-mg/kg dose		Multiple doses[b]	
	M	F	M	F	M	F
Mancozeb[c]	7.5	8.5	9.0	8.8	8.3	7.3
Unknowns 1-3	0.6	0.3	1.9	1.8	0.6	0.6
EBIS	1.0	1.2	1.7	1.7	1.1	1.2
ETU	3.2	2.4	11.2	12.6	3.2	4.0
Unknown 4	--	--	2.5	2.5	--	--
EU	1.9	2.0	5.3	4.9	2.1	4.0
Unknown 5	--	--	2.0	2.1	--	--
N-AcEDA	4.2	4.4	2.2	3.1	6.3	8.2
(N-ForEDA)[d]	--	--	2.0	2.0	--	--
(N-AcGly)[d]	4.4	5.6	5.7	6.7	5.4	8.0
(Gly)[d]	3.7	4.7	2.6	2.6	2.2	3.2
EDA	2.1	2.4	4.0	4.4	2.1	3.2
Origin	7.1	6.4	10.4	9.9	4.8	6.6
Second Extract	38.2	31.4	--	--	30.2	30.7
Not Extracted	22.0	30.9	31.1	27.2	29.0	16.9
Total	96.0	95.3	91.6	90.4	94.0	91.2

[a] Values are the means of three determinations.

[b] Animals administered a single oral dose of [^{14}C]mancozeb at 1.5 mg/kg after 2 weeks on a diet containing technical mancozeb at 15 ppm active ingredient.

[c] Determined as EBDC by HPLC.

[d] Tentatively identified by TLC only.

TABLE 6. Metabolite Profile in Urine from Male (M) and Female (F) Rats Dosed with [^{14}C]Mancozeb

Metabolite/ Degradate	Percent of [^{14}C] in Urine[a]					
	1.5-mg/kg dose		100-mg/kg dose		Multiple doses[b]	
	M	F	M	F	M	F
EBIS	1.5	1.8	1.4	1.4	1.5	0.8
ETU	36.9	35.5	40.0	42.7	30.8	35.9
Unknown 4	8.5	4.3	3.9	4.4	7.2	5.1
EU	8.1	7.5	13.4	12.7	8.6	8.6
N-AcEDA	7.7	6.2	9.6	9.9	13.0	15.0
(N-ForEDA)[d]	9.8[c]	10.6[c]	3.1	2.5	7.5	2.4
(N-AcGly)[d]			4.4	3.8	2.8	5.2
(Gly)[d]	4.6	6.3	6.4	5.2	4.3	5.3
EDA	7.7	7.7	4.9	6.2	6.4	5.2
Origin	7.6	8.0	6.4	5.9	8.8	9.8
Total	92.4	87.9	93.5	94.7	90.9	93.3

[a] Values are the means of three determinations.

[b] Animals administered a single oral dose of [^{14}C]mancozeb at 1 mg/kg after 2 weeks on a diet containing technical mancozeb at 15 ppm active ingredient.

[c] These were not separated.

[d] Tentatively identified by TLC only.

The metabolite/degradate profile of bile from rats dosed with [^{14}C]mancozeb is presented in Table 7. Major metabolites included the nonpolar unknowns 1-3 (although it is not certain how many compounds are in this fraction), ETU, EDA, and the metabolite tentatively identified as N-AcGly.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Approximately half of an oral dose of mancozeb was absorbed in rats. Results suggested that the absorption, distribution, and elimination of [^{14}C] was nonlinear for high (100 mg/kg) and low (1.5 mg/kg) [^{14}C]mancozeb-dosed rats. Absorption of [^{14}C]-mancozeb-derived radioactivity was moderately rapid; peak [^{14}C] levels were achieved within 3 and 6 hours after administration of 1.5 and 100 mg/kg [^{14}C]mancozeb, respectively. Elimination of radioactivity from the plasma was biphasic; the elimination half-lives for the rapid and slow elimination phases were 3.9 to 6.1 and 23.1 to 38.5 hours, respectively [rapid phase was much less pronounced in high-dose (100 mg/kg) rats]. Most of the [^{14}C]mancozeb dose was eliminated in the excreta within 24 hours; the eliminated dose was essentially evenly divided between the urine and feces. Most of the radioactivity eliminated in the feces represented unabsorbed material since only 2.0 to 8.8 percent of the dose was excreted in the bile. Thyroid tissues contained the greatest [^{14}C] concentrations after oral [^{14}C]mancozeb treatment. Peak thyroid [^{14}C] concentrations were not proportional to dose and were disproportionately less than the respective peak blood [^{14}C] concentrations after 100 mg/kg [^{14}C]mancozeb than after 1.5 mg/kg [^{14}C]mancozeb, indicating saturation at the high dose. Pretreatment with dietary nonradiolabeled mancozeb did not significantly alter the disposition or excretion of [^{14}C]mancozeb.

The estimated bioavailability of ETU in rats following oral administration of mancozeb represents only 3.1 to 6.4 percent of the absorbed [^{14}C]mancozeb-derived radioactivity. ETU in thyroid, liver, and plasma comprised only a small portion of the total mancozeb-derived material present in these tissues; and ETU was rapidly eliminated from these tissues (t 1/2 = 3.9 to 4.7 hours). EBDC was detected in liver, but not in thyroid of rats following oral mancozeb administration.

The metabolism of mancozeb was extensive and complex. Metabolites other than ETU were generally present in low amounts and were of a highly polar nature. The degradates ETD, EBIS, ETU, and EDA can arise via chemical conversion of mancozeb (EU can be derived from chemical conversion of ETU). Whether these compounds are truly metabolites rising from enzymatic conversion is doubtful. However, N-AcEDA, Gly, and N-AcGly are metabolites, most likely from metabolism of EDA. A proposed degradation metabolite pathway scheme is depicted in Figure 8 (see Figure 1 for structures). The metabolites arising from EDA are complex and lead to incorporation of [^{14}C] into the carbon pool.

TABLE 7. Metabolite Profile in Bile from Male (M) and Female (F) Rats Dosed with [^{14}C]Mancozeb

Metabolite/Degradate	Percent of [^{14}C] in Bile[a]			
	1.5-mg/kg dose		100-mg/kg dose	
	M	F	M	F
Unknowns 1-3	1.8	1.3	8.5	15.3
EBIS	2.6	2.1	3.2	2.5
ETU	3.7	4.1	11.5	14.5
EU	6.3	8.0	5.1	6.5
N-AcEDA	5.9	6.0	6.0	6.4
(N-AcGly)[b]	8.9	9.9	11.2	11.0
(Gly)[b]	28.2[c]	32.2[c]	6.7	6.8
EDA			12.1	11.4
Origin	30.9	24.2	20.1	17.0
Total	88.3	87.8	84.4	91.4

[a]Values are the means of three determinations except for male rats dosed at 1.5 mg/kg where n = 2.

[b]Tentatively identified by TLC only.

[c]Metabolites were not separated.

Unmetabolized EBDC was detected in the feces by HPLC, though not to a great extent (6.4-12.3 percent). However, CS_2 analysis of the feces indicated that 28.2-46.7 percent (low- and high-dose groups only) of the [^{14}C] activity was EBDC. The discrepancy may result from binding of mancozeb to the feces and/or continued degradation of mancozeb in the feces. More importantly, these results indicate that an extensive amount of [^{14}C] activity in the feces was associated with EBDC, regardless of whether EBDC was intact or degraded at the time of analysis. The metabolism of EBDC may be different at the different dose levels. Although with regards to chronic toxicity, the low-dose level represented the no-effect level and the high-dose level represented a minimum-effect level, it is interesting to note that the amount of ETU was significantly higher in the high-dose group in both the feces and bile.

B. A quality assurance statement was included with the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The metabolism of mancozeb is very complex. This complexity appears to be due to mancozeb readily degrading to a number of compounds. Furthermore, the study authors state that standard extraction procedures can cause mancozeb to degrade, thereby confounding the metabolite profile obtained from tissue or sample extracts. A second factor contributing to the difficulty of the problem is that mancozeb is degraded/metabolized to numerous compounds, most of which are highly polar and, therefore, difficult to isolate and identify. There is also evidence that the [^{14}C]ethylene carbons of [^{14}C]mancozeb enter the general carbon pool, making them difficult to isolate.

The study authors have taken notice of these problems and have attempted to develop methods for overcoming them. The methodology used and the data generated by these studies produced results that were consistent within study groups as well as in agreement with previously published reports on the metabolism of mancozeb and related compounds. We assess that, in general, the study authors' conclusions are well supported by the results and do take into consideration the limitations discussed above.

An important limitation to accurately portraying the metabolism of mancozeb that the authors have not fully discussed is the impurities found in the test material. HPLC analysis of the [^{14}C]mancozeb indicated the presence of 8-12 percent radiolabeled impurities, yet the study authors did not report the identities of these impurities. Therefore, the contribution of these [^{14}C] contaminants to the reported kinetics and metabolite profiles is entirely unknown. Other minor deficiencies that may have had some effect on the study results are: 1) animals dosed at 1.5 and 100 mg/kg were born on the same day but were dosed 2 weeks apart, resulting in as much as a 2-fold weight difference between groups (the low-dose animals were older and

heavier); 2) total recoveries of [^{14}C] were quite high, especially for high-dose females where the recovery for one animal was 142 percent of the dose, indicating errors in dosing and/or recovery procedures, and 3) the percent of dose excreted in the bile of the 1.5-mg/kg dose groups was based on data from only one male and two female rats due to unexplained "technical difficulties."

The only conclusions that the study authors made that we feel are not strongly supported by the data are: 1) proportionally higher amounts of ETU were excreted in the feces of high-dose animals than low-dose ones and 2) the data show that ETU levels have plateaued at 100 mg/kg. Both conclusions may be correct, but the first is questionable because metabolite analyses of fecal samples from high-dose animals were performed on both EDTA/ethanol and ethanol/water extracts whereas metabolite analyses of fecal samples from low-dose animals were performed on EDTA/ethanol extracts only. The second conclusion, which is indirectly supported by much of the data, cannot be made, because more than two dose levels are required to investigate the point at which [^{14}C]ETU levels would plateau.

Item 15--See footnote 1.

16. CBI APPENDIX: Appendix A, Methodology for the Isolation, Identification, and Quantitation of [^{14}C]mancozeb Metabolites/ Degradates in Rat Excreta; Appendix B, Study Protocol.

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TOXICOLOGY BRANCH: DATA REVIEW

005425

Chemical: Mancozeb

Caswell: 913A
EPA Chem.#: 014504

Study Type: Teratology - rabbit.

Citation: Somers Test on Dithane M-45, by J.R. Brown, Head, Hygiene Dept., U. Toronto (1968)

Accession No./MRID No.: N/A MRID - 1MM207

Sponsor/Testing Lab.: R&H/Brown

Study No./Date: N/A (August 8, 1968) 65-RC-1013

Test Material: Dithane M-45 technical (80% ai) dissolved in corn oil for oral intubation.

Procedures:

Three groups of ten NZ White female rabbits each were repeatedly mated (at least four times), then administered test material by oral intubation on D-7 through D-16 of presumed pregnancy at levels of 0 (corn oil), 25 and 250 mg/kg/day. One-half of each group was sacrificed on D-29 (prior to birth) and fetuses examined for soft tissues and skeletal abnormalities. The remainder of each group was allowed to go to term; litters weighed at weaning (21 days post-term), and pups examined for gross and histological anomalies. Susceptibility to teratogenic agents was ascertained in a fourth group injected IM daily with 5 mg/kg 6-mercaptopurine (6-MP) and sacrificed on D-29 of gestation.

Results:

Except for a slight decrease in maternal body weight at 250 mg/kg, no maternal or fetotoxicity was observed, and no gross or histological developmental abnormalities found in treated offspring, either from cesarean section or postnatally. In contrast, 6-MP treated fetuses exhibited limb atresia.

Conclusions:

The study concluded that 25 and 250 mg/kg/day mancozeb did not produce fetal abnormalities.

TB Evaluation/Core:

SUPPLEMENTARY: The teratology phase was not tested in a sufficient number of rabbits per dose group. The registrant is currently performing a new teratology study in rabbits.

005425

Maternal NOEL = 25 mg/kg/day
LEL = 250 mg/kg/day (decreased body weight)

Fetal NOEL > 250 mg/kg/day (HDT)
Teratogenic NOEL > 250 mg/kg/day (HDT)

A/D Ratio = $\frac{\text{Maternal NOEL}}{\text{Fetotoxic NOEL}} = \frac{25}{250} = 0.1$

Irving Mager, Ph.D. 8-27-86
Toxicology Branch
Hazard Evaluation Division (TS-769C)

J.E.K. 8/27/86

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TOXICOLOGY BRANCH DATA REVIEW

005425

CHEMICAL: Mancozeb

Caswell No.: 913A
EPA Chem No.: 014504

STUDY TYPE: Acute Studies (Oral LD_{50}, Dermal LD_{50}, Skin/Eye Irritation)

CITATION: Toxicity Report on Dithane M-45 DG Fungicide: Final Report, 83R-086A

ACCESSION No.: N/A

MRID: 142522

SPONSOR: Rohm & Haas Company, Philadelphia, PA

TESTING Lab.: Toxicology Department, Rohm & Haas

STUDY NO.: 83R-086A

DATE: June 20, 1983

Test Material:

Dithane M-45 technical, TD #83-58 (Lot WHC-0651), 72.6% ai, a brownish-gray solid, dispersed in various vehicles for testing.

Procedures:

[A] Acute Oral LD_{50} - Rat: Ten male and ten female Sprague-Dawley (Charles River CRCD) rats were gavaged once with 5000 mg/kg test material dispersed in .5% methocel (dosage volume= 20 ml/kg) and observe[illegible]

[B] Acute Dermal LD_{50} - Rabbit: The dorsum of [illegible] six female New Zealand (NZ) White (Dutchland Farms) rabbits were clipped of hair (and the skin of 1/2 of each sex abraded), and a 1:1 saline paste of test material (5000 mg/kg) held in place under an impervious cuff for 24 hours. The dorsum was then gently wiped of test material and the animals observed for 14 days.

[C] Primary Skin Irritation - Rabbit: A 1:1 saline paste containing 500 mg test material was applied to the skin of six male NZ White rabbits, and held in place under an impervious cuff for 4 hours, following which the treated area was gently wiped free of material, and the animals observed for 7 days.

22[illegible]

[D] Primary Eye Irritation - Rabbit: 100 milligrams of test substance were instilled into the eyes of nine NZ White rabbits, three of which were washed 20 to 30 seconds after dosing, following which all animals were observed for 7 days. Score was according to Draize (1944).

Results and Conclusions:

[A] Oral LD_{50}: No animals died. Except for mild constipation ("scamt droppings"), tan-stained muzzle and brown-stained anogenital area beginning on the day of dosing and persisting into the second week, no gross changes were recorded at necropsy. The authors conclude the oral LD_{50} of the test material is greater than 5000 mg/kg in both males and females.

[B] Dermal LD_{50}: No animals died. Except for constipation ("scamt droppings") in all animals, no gross changes were recorded. The authors conclude the dermal LD_{50} of the test material is greater than 5000 mg/kg.

[C] Skin Irritation: The mean PIS at 72 hours (= the sum of mean erythema and mean edema scores) was calculated as 0.2, i.e., slightly irritating, and 0 (zero) thereafter.

[D] Eye Irritation: The following mean Draize values were calculated for the six unwashed eyes:

Time After Dosing	Cornea	Iris	Conjunctivae
1 hr	3.3	0.8	12.3
3 hr	1.7	0.0	3.3
72 hr	0.0	0.0	2.3
7 da	0.0	0.0	0.0

The authors conclude the test substance is a moderate ocular irritant up to 72 hours, but all ocular effects were reversible within 7 days.

TB Evaluation/Core:

Test	Reported Results	Core	Tox. Cat
[A] Acute Oral	LD_{50} > 5000 mg/kg (males/females)	Minimum	IV
[B] Acute Dermal	LD_{50} > 5000 mg/kg (males/females)	Minimum	III
[C] Skin Irritation	PIS = 0.2 (72 hr)	Minimum	IV
[D] Eye Irritation	Draize = 2.3 (72 hr)	Minimum	III

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division
Date: 08/27/86

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89114:Mauer:HED-05:KENCO:8/20/86:9/1/86:dej:VO
R:89118:Mauer:HED-05:KENCO:8/26/86:9/5/86:NeeCee

005425

TOXICOLOGY BRANCH DATA REVIEW

CHEMICAL: Mancozeb

Caswell No.: 913A
EPA Chem No.: 014504

STUDY TYPE: Acute Studies (Oral LD_{50}, Dermal, Inhalation)

CITATION: Acute Toxicity Tests with Zimaneb

ACCESSION NO.: N/A

MRID: 047146

SPONSOR: (Not stated)

TESTING LAB: University of Miami, School of Medicine (M.L. Keplinger)

STUDY NO.: (Not stated)

DATE: (Not stated, but received at EPA January 11, 1965)

Test Material:

Technical, stated as 80 percent (the nature and/or other descriptors not provided).

Procedures:

[A] Acute Oral - Rat: A 25 percent suspension of test material in CMC was administered once by gavage to groups of male and female Osborne-Mendel rats (generally five to six of each sex) at seven doses ranging from 940 to 10,700 mg/kg; animals were observed for 14 days, weighed 7 and 14 days postdose, and food consumptions recorded daily. All animals were autopsied either at death, or survivors at termination.

[B] Acute and Subacute Dermal - Rabbit: Undiluted test material was applied once at 10 g/kg ai to the clipped abdomen of two male and two female rabbits (strain unstated) and left in contact with the skin for 8 hours. A further four males and four females were treated at 5 g/Kg daily for 5 days. The skin of half of each group was abraded before application. All animals were observed for a total of 14 days from the initial treatment.

[C] Primary Skin Irritation: Two rabbits (one male, one female) were patch-tested using 2 grams of the substance applied by a soaked 1 x 1-in gauze pad taped under a

larger sponge to intact skin clipped free of hair, and left in place for 3 days. Treated skin was examined daily.

[D] Acute Inhalation - Rat: Osborne-Mendel rats (six males, six females) were exposed for 4 hours to the test material as a dust (whose initial large particle size was reduced by grinding), stated to be "a virtual 'cloud' . . . which literally covered the animals," at a nominal concentration of 6.85 mg/L (= 6.85 g/m^3), and observed for 14 days.

Study Results and Conclusions:

[A] Deaths occurred in a dose-related manner in rats treated at levels of 3200 mg/kg and above, generating a combined LD_{50} of 4500 mg/kg (3600-5700 mg/kg), according to the method of Deichman and LeBlance (1943). Toxicity appeared several hours after administration (dyspnea, hemorrhagic rhinitis, excessive salivation, and clay-colored stool), and deaths approximately 18 hours after a lethal dose. Weight loss averaging 8 grams in females and 14 grams in males occurred within the first week, but treated animals recovered their initial body weight during the second week; this loss appeared to be correlated with food consumption (less during the first 7 days postdose, and recovery during the ensuing week). Serous fluid within the pleural cavity, gas in the stomach, and clay-colored masses in the large intestine were constant findings in animals that died; no gross changes, however, were found in term animals.

[B] No clinical effects or gross findings were recorded after either acute or repeat dermal administration of test compound.

[C] No effect on rabbit skin (i.e., no primary irritation) treated with undiluted test material was observed at 24, 48, or 72 hours.

[D] Except for "very slight irritation to the nose and mucous membranes" of treated rats, no other clinical effects were reported. Autopsy of two rats (sex not stated, but presumably, one male and one female) 2 hours after exposure and two more at 24 hours revealed only "mild diffuse congestion in the lungs" but no other gross findings in (unstated) organs or tissues. The organs and tissues of the remaining rats sacrificed at term were stated to be normal.

TB Evaluation/Core:

Study Type	Reported Results	Core	TOX CAT.
[A] Acute Oral - Rat	LD_{50} (combined sexes) = 4500 mg/kg (3600-5700 mg/kg)	Minimum	III
[B] Acute Dermal - Rabbit	LD_{50} > 10,000 mg/kg (ODT)	Minimum	III
Subacute Dermal - Rabbit	5000 mg/kg/day (ODT) for 5 days had no clinical effect		---
[C] Primary Skin Irritation - Rabbit	No irritation from treatment with 2 g (ai)	Supplementary[1]	IV
[D] Acute Inhalation - Rat	LC_{50} > 6.85 mg/L	Supplementary[2]	IV

1 Too few animals tested.
2 Actual concentration and respirable particle size not determined.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division
Date: 9/05/82

REVIEWER

005425

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

AUG 27 1986

MEMORANDUM

SUBJECT: Mancozeb - Data Submitted in Response to Data Call-In Notice of January 17, 1983 ID No. 707-78 - Accession No. 259044

Caswell No.: 513A

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Arvella Farmer, PM 65
Special Review Branch
Registration Division (TS-767C)

THRU: Jane E. Harris, Ph.D., Head
Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Rohm & Haas
Philadelphia, PA

Action Requested (870):

Review and evaluate studies submitted in response to the Data Call-In Notice of January 17, 1983.

TB Conclusions:

In addition to twelve (12) mutagenicity assays which have already been evaluated (see attached cover memorandum: Mauer to Farmer, dated May 8, 1986), three acute oral studies

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were included with this submission (Accession No. 259044), but were not reviewed at that time, namely (as listed in the registrant's Table of Contents):

Section 2. Acute Oral LD_{50} in B6C3F1 Mice With Dithane M-45 Fungicide (R & H Report No. 83R-213A, dated September 24, 1984).

Section 3. Acute Oral LD_{50} in Fischer-344 Rats With Dithane M-45 Fungicide (R & H Report Nos. 83R-213B and 83R-0218, dated, respectively, September 21 and 24, 1984).

These additional studies have now been evaluated for inclusion in the Mancozeb Registration Standard, as follows:

Study No.	Reported Results	TB Core	Tox Cat
83R-213A: Mice	LD_{50} > 5 g/kg	Supplementary	IV
83R-213B: Rats	LD_{50} > 5 g/kg	Supplementary	IV
83R-0218: Rats	LD_{50} > 5 g/kg	Supplementary	IV

Individual Data Reviews are attached to this memorandum

cc: Jacoby/Cool, PM 21
Registration Division (TS-767C)
Judy Hauswirth, Coordinator
Toxicology Branch
Hazard Evaluation Division (TS-769C)
Susan Lewis,
Special Review Branch
Registration Division (TS-767C)

005425

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Mancozeb

Caswell: 913A
EPA Chem: 014504

Study Type: Acute oral LD_{50} - Mice

Citation: Acute Oral LD_{50} in B63CF1 Mice with Dithane M-45 Fungicide

Accession No.: 259044

MRID: N/A (IM-0001)

Sponsor: Rohm & Haas Co., Philadelphia, PA.

Testing Lab.: Toxicology Department, Rohm & Haas.

Study No.: 83R-213A

Date: September 24, 1984.

Test Material:

Dithane® M-45, TD No. 83-224 (Lot 0842), technical, 80 percent ai, a buff-yellow powder, dispersed in corn oil for oral administration.

Procedures:

Groups of 10 male mice (Charles River B6C3F1) were gavaged once orally at 0 (10 mL/kg corn oil) and 5000 mg/kg test material, and observed for 14 days.

Results:

No mice died. Three test and five control animals had transient yellow/brown-stained anogenital areas shortly after dosing, but no other gross changes in appearance, behavior or body weight were recorded during the observation period.

Study Conclusions: The acute oral LD_{50} > 5000 mg/kg.

TB Evaluation/CORE:

CORE-SUPPLEMENTARY. Females were not tested. Acute Oral LD_{50} (males) > 5000 mg/kg. TOX CAT IV.

Irving Mauer Ph.D. 10/23/84
Toxicology Branch
Hazard Evaluation Division (TS-769C)

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TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Mancozeb

Caswell: 913A
EPA Chem: 014504

Study Type: Acute Oral LD_{50} - Rats

Citation: Acute Oral LD_{50} in Fischer 344 Rats with Dithane M-45 Fungicide.

Accession No.: 259044

MRID: N/A (IM-0002)

Sponsor: Rohm & Haas Co., Philadelphia, PA.

Testing Lab.: Toxicology Department, Rohm & Haas.

Study No.: 83R-213B

Date: September 24, 1984.

Test Material:

Dithane® M-45, TD 83-224 (Lot No. 0842), technical, 80 percent ai, a buff-yellow powder dispersed in corn oil for oral administration.

Procedures:

Groups of 10 male F-344 rats (Charles River) were gavaged once at 0 (10 mL/kg corn oil) or 5000 mg/kg test material and observed for 14 days.

Results:

No animals died. One test animal developed persistent alopecia beginning 3 days postdose (still present at autopsy), and five controls showed transient yellow/brown stained anogenital areas shortly after dosing, but recovered on the second day. No other gross changes in appearance or behavior were recorded, and both groups gained weight during the 14-day observation period.

Study Conclusions: The acute oral LD_{50} > 5000 mg/kg.

005425

TB Evaluation/CORE:

CORE-SUPPLEMENTARY. Females were not tested. Acute oral LD_{50} (males) > 5000 mg/kg. Tox. Cat. IV.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

08-21-86

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Mancozeb

Caswell: 913A
EPA Chem: 014504

Study Type: Acute Oral LD_{50} - Rats

Citation: Acute Oral LD_{50} in Fischer 344 Rats with Dithane M-45 Fungicide.

Accession No.: 259044

MRID: N/A (IM-0002)

Sponsor: Rohm and Haas, Philadelphia PA

Testing Lab.: Toxicology Department, Rohm and Haas.

Study No.: 83R-218

Date: September 21, 1984.

Test Material:

Dithane® M-45 technical (TD 83-244, Lot No. 0842), 80 percent ai, a buff-yellow powder suspended in distilled water for oral administration.

Procedures:

Ten male F-344 rats (Charles River) were gavaged with 5000 mg/kg test material and observed for 14 days.

Results:

No animals died. All except two animals showed one or more of the following signs immediately after dosing, persisting into the second week of the observation period: passiveness (2/10), respiratory noise (1/10), abdominal breathing (1/10), lacrimation (1/10), red-stained eyes (2/10), red-stained muzzle (2/10), tan-stained muzzle (6/10), yellow-stained anogenital area (4/10). All, however, had recovered by necropsy, and no body weight loss was recorded.

Study Conclusions: Acute oral LD_{50} > 5000 mg/kg.

TB Evaluation/CORE:

CORE-SUPPLEMENTARY.

No females were tested, and no controls run. Acute oral LD_{50} (males) > 5000 mg/kg. Tox. Cat. IV.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

08-25-86

005425

TOXICOLOGY BRANCH DATA REVIEW

Chemical: Mancozeb

Caswell No.: 913A
EPA Chem No.: 014504

Study Type: (86-1) Subchronic Oral Toxicity (Feeding) - Chicken

Citation: Subacute (8-Week) Toxicity Study with the Fungicide Dithane M-45 in Chickens

Accession No.: N/A

MRID No.: 129288

Sponsor: MINOC S.A.R.L. (Paris, France)

Testing Lab.: Centraal Instituut Voor Voedingsonderzoek (The Netherlands), A. Engel and H. van der Meulen

Study No.: NR. R-3290

Date: October 1970

Test Material:

Dithane M-45 (although unstated, presumably the technical, said to be ". . . based on ethylene bisdithiocarbamate, and containing 16% manganese and 2% zinc").

Procedures:

Four groups of 15 male and 15 female 1-day-old "broiler chickens" (from Hybro, but strain unstated) were fed test material in their diet at levels of 0, 0.02%, 0.1%, and 0.2% = 0, 200, 1000, and 2000 ppm) for 8 weeks. The birds were weighed and food consumption determined weekly, and hematological (hb, pcv, rbc) values measured at week 7. At termination, all birds were examined for gross pathological changes, liver and thyroids weighed, and tissue samples of these organs as well as additional organs (kidneys, brain, gi tract, femoral nerve) prepared for histological study.

Results:

Three male and two female high-dose birds as well as one mid-dose male died or were sacrificed *in extremis* following severe paralysis of the legs (time into study not stated). Compared to controls, high-dose males and females, as well as mid-dose males, gained

less body weight during the 8-week treatment period, and high-dose animals averaged less food consumption. The selected hematological values were comparable in all groups. Dose-related highly significant increases in relative weight of thyroids were recorded at the two higher levels (0.1%, 0.2%); in addition, slight but significant increases in liver-to-body weight ratios were recorded for mid- and high-dose males, but not females. Histopathological changes were observed only in thyroids, consisting of follicular enlargement and distention by "abundant amount of colloid," fused/cystic irregular follicles. These goitrous changes were most pronounced in high-dose birds of both sexes, but were also present in a few birds treated at 0.1% mancozeb. (However, no detailed analyses of individual birds nor any tabulated summary of these changes were presented.) Non-compound-related changes in liver (periportal inflammatory infiltration) and kidneys (focal tubular nephrosis) were also found (i.e., stated to be of the same degree and frequency in both controls and treated birds), but again no individual animal or summary data were presented.

Study Conclusions:

Levels of 0.1% and 0.2% (but not 0.02%) Dithane M-45 fed to chickens caused deleterious effects, principally thyroid enlargement accompanied by colloid goitrous lesions, but also leg paralysis, decreased growth in males at the mid-dose and females plus males at the high-dose, and increased liver weight in males.

TB Evaluation/Core:

Supplementary Data. No individual animal data; no reporting on neural histology.

NOEL = 0.02% (200 ppm).

LEL = 0.1% (1000 ppm); leg paralysis, [illegible] in weight gain in males, increas[illegible] thyroid weight in both sexes, relative liver weights in males, and goiter with colloid enlargement in both sexes.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)
Date: 9/11/86

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

005425

MAY 8 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mancozeb - Review of Mutagenicity Data Submitted on Mancozeb and ETU in Response to Data Call-In Notice of January 17, 1983 - ID #707-78, Accession No. 259044

Caswell No. 913A

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C) 04-30-86

TO: Arvella Farmer, PM 65
Special Review Branch
Registration Division (TS-767C)

and

Henry M. Jacoby, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Jane E. Harris, Ph.D. 4/30/86
Head, Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769C)
5/1/86

Registrant: Rohm & Haas

Action Requested:

(870) Review and evaluate the following mutagenicity studies in response to the Data Call-In Notice dated January 17, 1983. Individual Data Reviews are attached to this memorandum.

Study Title (No. and Date)	Reported Result	Reviewer Evaluation
Mammalian Cell Transformation Assay in C3H 10T 1/2 Cells with Ethylenethiourea (No. 84R-056, November 19, 1984).	Negative	Acceptable
Mammalian Cell Transformation Assay for Promotion in C3H 10T 1/2 Cells with Ethylenethiourea (No. 84R-298, May 29, 1985).	Negative	Unacceptable
Microbial Mutagen (Ames) Test with Dithane M-45 Fungicide (S-9 prepared from Aroclor 1254-induced Fischer 344 rats) (No. 84R-0059, June 21, 1984).	Negative	(Incomplete)*
Microbial Mutagen (Ames) Test with Dithane M-45 Fungicide (S-9 prepared from Aroclor 1254-induced B6C3F1 mice) (No. 84R-0060, June 21, 1984).	Negative	(Incomplete)*
Host Mediated Assay in Mice with Dithane M-45 Fungicide (No. 84RC-25B, September 26, 1984).	Negative	Unacceptable
Host Mediated Assay in Mice with Dithane M-45 Fungicide (Repeat Study) (No. 84RC-48, July 1, 1985).	Negative	Acceptable
Gene Mutation Assay in CHO Cells with Dithane M-45 Fungicide (No. 84R-207, February 11, 1985).	Negative	(Incomplete)*
Unscheduled DNA Assay in Rat Hepatocytes with Dithane M-45 Fungicide (No. 84R-280, May 29, 1985).	Negative	Inconclusive**
Cytogenetic Study in Fischer-344 Rats with Dithane M-45 Fungicide (No. 84R-246, December 21, 1984).	Negative	Acceptable
Sister Chromatid Exchange Assay in CHO Cells with Dithane M-45 Fungicide (No. 84RC-60, March 1985).	Positive	Acceptable
Mammalian Cell Transformation Assay in C3H 10T 1/2 Cells with Dithane M-45 Fungicide (No. 84R-055, November 19, 1984).	Negative	Acceptable
Mammalian Cell Transformation Assay for Promotion in C3H 10T 1/2 Cells with Dithane M-45 Fungicide (No. 84R-297, May 29, 1985).	Negative	Unacceptable

* Acceptable with S-9 activation. Although initially declared "unacceptable under nonactivated conditions" because no positive controls were included for that part of the assay, the sensitivity of the test system to respond was demonstrated adequately in the activated assays. Hence these studies are ACCEPTABLE.

** Presumptively positive; procedural problems indicate assay should be repeated.

Two additional studies were included with this submission (Accession No. 259044), but were not reviewed at this time, namely (as listed in the registrant's Table of Contents):

"Section 2. Acute Oral LD50 in B6C3F1 Mice With Dithane M-45 Fungicide" (R & H Report No. 83R-213A, dated September 24, 1984).

"Section 3. Acute Oral LD50 in Fischer-344 Rats With Dithane M-45 Fungicide" (R & H Report No. 83R-213B, dated September 24, 1984).

These two acute studies will be evaluated for inclusion in the MANCOZEB REGISTRATION STANDARD.

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005425

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-02-4225
DYNAMAC No. 009
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity

STUDY IDENTIFICATION: Mutagenicity Overview on the Pesticide Mancozeb.

REVIEWED BY:

I. Cecil Felkner, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-3-86

Nancy E. McCarroll, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-3-86

APPROVED BY:

William L. McLellar. Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellar
Date: 4-3-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irving Mauer
Date: 04-07-86

Jane Harris, Ph.D.
EPA Acting Section Head

Signature: Jane Harris
Date: 04-07-86 JH

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TEST CHEMICALS: Mancozeb; Dithane M-45; coordination product of zinc and manganese ethylene bis-dithiocarbamate, and ethylenethiourea (ETU), a principal metabolic derivative.

STUDY/ACTION TYPE: Overview--Registration Action.

MUTAGENICITY OVERVIEW ON THE PESTICIDE MANCOZEB

Introduction: Under FIFRA Guideline Subdivision F: Pesticide Assessment Guidelines: Hazard Evaluation - Human and Domestic Animals, dated 11-30-82, an overview (Section 80-1) is required for the various subdivisions of toxicology. "This subdivision details the toxicity data recommended to support the registration of pesticide products," and should meet the requirements of good laboratory practice (40 CFR Part 160), if applicable.

For each test substance, bioassays must be performed to assess the "potential to affect the qualitative or quantitative integrity of human genetic material." A battery of tests to assess mutagenicity is therefore required with the objectives of:

1. Detecting, with great sensitivity, the capacity of a test material to alter cellular genetic material.

2. Determining the relevance of genetic alterations to mammals.

3. Incorporating positive genetic findings into the risk assessments for heritable effects, carcinogenicity, and other possible health hazards.

There are three categories of genetic effects that must be addressed by the test battery:

1. Gene mutations.

2. Structural chromosomal aberrations.

3. Other mutagenic mechanisms (e.g., direct DNA damage, microtubule/spindle fiber inhibition) as deemed appropriate for the test material.

Mutagenicity data as required by 40 CFR Section 158.135 are submitted to support the registration of each manufacturing-use product and of certain end-use products. The assays are performed with the technical grade of each active ingredient in the product. The product should be tested in nonactivated and metabolically activated in vitro assays, and should also be assayed using in vivo mammalian systems with all appropriate positive and negative controls.

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SUMMARY OF STUDY EVALUATIONS:

Category 1: Gene Mutation. There were five unpublished studies submitted by the registrant and one published report submitted by the reviewer which were in this category. They are summarized in Table 1 and discussed in this section according to assay type.

Salmonella typhimurium/microsome (Ames): Report Nos. 84R0059 and 84R0060 stated that Dithane M-45 did not induce mutations in any tester strains with or without S9 activation in a dose range of 2.5 to 250 µg/plate. The assays using S9 were conducted properly and therefore considered acceptable; however, the nonactivated assays performed in these studies did not include direct-acting positive controls and were not considered acceptable.

Host-mediated assay in mice (Salmonella typhimurium TA1530): Report No. 84RC-258, using S. typhimurium TA1530 as the detector in a host-mediated assay in mice, showed that Dithane M-45 was not mutagenic when the host was dosed at 0.5 to 5 mg/kg. However, the doses were inadequate; therefore, the assay was considered unacceptable. Report No. 84RC-48 described the use of a dose range of 0.5 to 5 g/kg in an otherwise identical host-mediated assay. Dithane M-45 was also negative for a mutagenic response and the assay was considered acceptable.

Mammalian cell mutagenicity--CHO/HGPRT: Report No. 84R-207 stated that Dithane M-45 was not mutagenic in the CHO/HGPRT in vitro assay either at 0.5 to 15 µg/mL in the nonactivated assay or at 0.25 to 45 µg/mL in the S9-activated assay. The S9-activated assay was properly conducted and therefore acceptable; however, the nonactivated assay was unacceptable because the positive control, ethylmethane sulfonate, had to be used at a level that was approximately 7-fold higher than the highest nonactivated dose, 15 µg/mL of test material.

Published data on Bacillus subtilis and Salmonella typhimurium (liquid preincubation) assays: Using a dose range of 1 to 25 µg/plate in the liquid preincubation assay with B. subtilis and S. typhimurium, Shiau et al. (1980)[1] reported that Dithane Technical (zinc ethylenebisdithiocarbamate) was mutagenic in both B. subtilis TKJ6321 and S. typhimurium TA1535. Although mutagenesis in the Salmonella mutant was not as strong as in B. subtilis, it could be demonstrated at nonactivated concentrations of 1, 5, and 10 µg/plate. In B. subtilis TKJ6321, a dose-responsive increase was observed in the nonactivated system at this same dose range, with a decline due to cytotoxicity at 25 µg/plate. At the highest concentration, approximately 45,000 His^+ revertants/10^8 cells was reported, and the calculated potency was 38.4 revertants/nanomole. In the presence of S9, Dithane was reported to show a "marked reduction" in the mutagenic response.

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Overall, the unpublished reports showed that Dithane M-45 was not mutagenic either in vitro using bacteria and mammalian cells with or without S9 activation, or in the host mediated assay. However, none of the assays were acceptable for the in vitro nonactivated system. Since published data using the liquid preincubation nonactivated system for both B. subtilis and S. typhimurium were positive, and in fact showed that Dithane was a very potent mutagen, inducing 38.4 revertants/nanomole, the test material should be considered mutagenic. It is classified as a base-pair mutagen on the basis of its positive activity for S. typhimurium strain TA1535 and B. subtilis strain TKJ6321. If all the unpublished and published data are considered, there are no data gaps for Category 1.

Category 2: Structural Chromosomal Aberrations.

Sister chromatid exchange: Study No. 84RC-60 reported that Dithane M-45 caused an increase in sister chromatid exchange in CHO-WB1 cells which was dose responsive in two assays in the range of 7.5 to 17.5 µg/mL in the nonactivated system (positive response beginning at 7.5 µg/mL), and inconclusive but presumed positive in the range of 10 to 17.5 µg/mL in the S9-activated assay (positive response beginning at 10 µg/mL). The assays were conducted properly and, therefore, acceptable.

In vivo cytogenic study in rats: The report No. 84R-246 stated that Dithane M-45 did not cause a significant increase in chromosomal aberrations in bone marrow cells of male rats exposed to 4.4 g/kg (maximum tolerated dose) sampled over the entire mitotic cycle. This data was acceptable; however, the study did not include female animals and was, therefore, deficient.

Except for the data gap on chromosomal aberrations in female animals, there is sufficient data to fulfill the requirements in Category 2. Since a positive response was obtained in the SCE assay in the nonactivated system and a presumptive positive in the S9-activated system, we presume that the test material causes structural chromosomal aberrations and that the frequency of inducing these aberrations could be reduced in either the presence of an exogenous or endogenous mammalian metabolic system.

Category 3: Other Mutagenic Mechanisms.

Unscheduled DNA synthesis in rat hepatocytes: The report No. 84R-280 stated that Dithane M-45 did not induce unscheduled DNA synthesis in isolated Fischer rat hepatocytes. However, we conclude that the net nuclear grain counts at 1.0, 2.5, and 5.0 µg/mL, although complicated by a higher than usual cytoplasmic grain count for the solvent and a decrease at 10 µg/mL, were sufficient to presume a positive but inconclusive UDS response.

DNA-damage in Bacillus subtilis: Shiau et al. (1980)[1] published data showing that Dithane technical caused DNA damage in several repair-deficient strains of B. subtilis at 50 µg/plate in the nonactivated system, but that S9 metabolism reduced the effect so that 300 µg/plate was required for a positive response. This data is supported by the

thesis work of Lee[2] (1980) who demonstrated DNA damage in B. subtilis strains hcr-9, fh2006-7, and mc-1 in the nonactivated system at all doses between 50 and 300 µg/plate. In addition, a positive DNA-damaging effect in a liquid incubation assay was demonstrated at all doses from 10 to 50 µg/mL. In an attempt to define the mechanism of Dithane action, Lee also showed that the DNA derived from B. subtilis cultures treated with 10 µg/mL of Dithane was damaged, and therefore had reduced DNA transformation activity in the histidine gene; furthermore a 1:1 ratio of Dithane mixed with purified DNA, damaged the DNA and specifically reduced DNA transformation for the histidine locus.

Hume[3] (1980) reported that nonactivated Dithane was negative in the phage induction assay (β-galactosidase induction) in Escherichia coli B13 (λ+) when doses ranging from 50 to 300 µg/plate were assayed in a semiquantitative spot test, but it was weakly positive at 50 µg/mL in the liquid (tube) assay.

In vitro transformation assays in C3H/10T1/2 mouse fibroblasts: Report 85R-055 stated that Dithane M-45 at doses ranging from 0.05 to 0.5 µg/mL did not induce transformed foci in C3H/10T1/2 mouse fibroblasts; likewise, ethylenethiourea (ETU) was reported to be negative in this system at doses ranging from 100 to 1,000 µg/mL (Report 84-R-056). These studies were properly conducted and therefore acceptable. However, study Nos. 84R-297 and 84R-298, designed to assess promoter activity in C3H/10T1/2 mouse fibroblasts, were unacceptable because only one dose of Dithane M-45 (0.1 µg/mL) or ETU (333 µg/mL) was used.

SUMMARY TABLE:

A one-liner table has been included in this overview. It identifies the individual studies, specifies the dose range, presents the reviewers' evaluations, places each study in its proper category, and classifies the studies according to their acceptability.

CONCLUSION:

If all studies submitted by the registrant and published articles are considered, testing in all three genetic effect categories are fulfilled. Although there were many studies submitted in which negative responses were obtained, there is sufficient evidence which shows that Dithane is mutagenic. Since this test material was shown to induce base-pair mutations in B. subtilis and S. typhimurium,[1] induce sister chromatid exchange (study No. 84RC-60), presumably induce unscheduled DNA synthesis (study No. 84R-280), induce DNA damage in B. subtilis,[1,2] induce β-galactosidase synthesis in the E. coli P^{+} (λ+) system,[3] and directly interact with DNA of B. subtilis,[2] it must be considered to have a significant genotoxic potential. The negative responses in some of the in vitro assays could have been contributed, at least in part, by the problems of solubility; the test material is very insoluble in water, therefore, using DMSO as the solubilizer was required.[1,2,3] In

addition, it appears that the S. typhimurium/microsome assays (study Nos. 84R0059 and 84R0060) lacked sensitivity and perhaps even specificity for detecting a gene mutation response; however, liquid preincubation and the use of DMSO as a solubilizer appeared to improve the sensitivity of the assay.[1] The inconclusive result in UDS assays could also be attributed to a failure in solubilizing the test material. When the transformation assays were performed, there was no indication that either Dithane or ETU induced or promoted transformed foci; these results are not surprising considering that the test materials may not have reached the target site under the conditions of the assays. In addition, transformation is considered to be a multistage phenomenon and the requirements to cause cell transformation would likely be less probable than induction of a point mutation, DNA interaction, or a clastogenic effect.

Literature Cited

[1] Shiau, S. Y., R. A. Huff, B. C. Wells, and I. C. Felkner. Mutation Research 71(1980): 169-179.

[2] Lee, M. C. DNA Transformation and Mutagenesis Studies on Dithane with Bacillus subtilis Repair-Deficient Strains. M. S. Thesis at Texas Tech University, supported by EPA Contract CR806904-01, 1980, pp. 1-21.

[3] Hume, S. H. Screening Pesticides with a Colorimetric Phage Induction Assay. M. S. Thesis at Texas Tech University, supported by EPA Contract CR806904-01, 1980.

TABLE 1. One-Liner Summary Table of Mutagenicity Studies with Mancozeb (Dithane M-45) and Ethylenethiourea

Study / Lab / Study Date	Material*	Accession No.	Dose	Conclusions (Evaluations)	Genetic Effects Category	Classification
1. Mutagenicity--Reverse Mutation in Salmonella/Rohm and Haas Co./ 84R0059/6-21-84	Dithane M-45, TB No. 83-224, lot No. 0842, 80% a.i.	259044	2.5-250 µg/plate	Not mutagenic for S9-activated; data inadequate for nonactivated but negative	1	S9--acceptable; nonactivated--unacceptable
2. Mutagenicity--Reverse Mutation in Salmonella/Rohm and Haas Co./ 84R0060/6-21-84	Dithane M-45, TB No. 83-224, lot No. 0842, 80% a.i.	259044	2.5-250 µg/plate	Not mutagenic for S9-activated; data inadequate for nonactivated but negative	1	S9--acceptable; nonactivated--unacceptable
3. Mutagenicity--Host-mediated Assay-Salmonella in mice/ Hazelton Labs./84RC-258/9-26-84	Dithane M-45, 80% a.i.	259044	0.5-5.0 mg/kg	Inadequate dosage; negative result	1	Unacceptable
4. Mutagenicity--Host-mediated Assay-Salmonella in mice/ Hazelton Labs./84RC-48/1-1-85	Dithane M-45, 80% a.i.	259044	0.5-5.0 g/kg	Not mutagenic	1	Acceptable
5. Mutagenicity--CHO/HGPRT/ Rohm and Haas Co./84R-207/ 2-11-85	Dithane M-45, TB83-224, lot No. 0842, 80% a.i.	259044	0.25-45 µg/mL	Not mutagenic for S9-activated; data inadequate for nonactivated	1	S9--acceptable; nonactivated--unacceptable
6. Mutagenicity--Sister Chromatid Exchange in CHO/Litton Bionetics, Inc./84RC-60/3-85	Dithane M-45, TB83-224, lot No. 0842, 80% a.i.	259044	7.5-17.5 µg/mL (2 assays)	Mutagenic in nonactivated assay (7.5 µg/mL); presumed mutagenic (10 µg/mL) in S9-activated assay	2	Acceptable

*Dithane M-45 which is the Rohm and Haas technical coordination product of zinc and manganese ethylene bis-dithiocarbamate.

TABLE 1. One-Liner Summary Table of Mutagenicity Studies with Mancozeb (Dithane M-45) and Ethylenethiourea (continued)

Study / Lab / Study Date	Material	Accession No.	Dose	Conclusions (Evaluations)	Genetic Effects Category	Classification
7. Mutagenicity--In vivo Cytogenetics in Rats/Rohm and Haas Co./ 84R-246/12-21-84	Dithane M-45,* TB No. 83-224, lot No. 0842, 80% a.i.	259044	4.4 g/kg	Not mutagenic in male rats; not tested in female rats	2	Acceptable in male rats; unacceptable in female rats
8. Mutagenicity--In vitro Unscheduled DNA Synthesis/Rohm and Haas Co./ 84R-200/5-29-85	Dithane M-45,* TB 83-224 lot No. 0842, 80% a.i.	259044	0.025-10.0 µg/mL	Presumptive positive at 1.0, 2.5, and 5.0 µg/mL	3	Inconclusive
9. Mutagenicity--In vitro Transformation in C3H/10T 1/2 Mouse Fibroblasts/Rohm and Haas Co./ 85R-055/11-19-84	Dithane M-45,* TB 83-224 lot No. 0842, 80% a.i.	259044	0.05-0.5 µg/mL	Negative at all doses	3	Acceptable
10. Mutagenicity--In vitro Transformation in C3H/10T 1/2 Mouse Fibroblasts/Rohm and Haas Co./ 84R-056/11-19-84	Ethylenethiourea, TB 83-223, lot No. 888-36, 99.8% pure	259044	100-1000 µg/mL	Negative at all doses	3	Acceptable
11. Mutagenicity--In vitro Transformation Assay for Promotion in C3H/10T 1/2 Mouse Fibroblasts/ Rohm and Haas Co./84R-297/ 5-29-85	Dithane M-45* TB 83-224, lot No. 0842, 80% a.i.	259044	0.1 µg/mL	Negative, but insufficient dose range	3	Unacceptable

*Dithane M-45 which is the Rohm and Haas technical coordination product of zinc and manganese ethylene bis-dithiocarbamate.

TABLE 1. One-Liner Summary Table of Mutagenicity Studies with Mancozeb (Dithane M-45) and Ethylenethiourea (continued)

Study / Lab / Study Date	Material	Accession No.	Dose	Conclusions (Evaluations)	Genetic Effects Category	Classification
12. Mutagenicity--In vitro Transformation Assay for Promotion in C3H/10T 1/2 Mouse Fibroblasts/ Rohm and Haas Co./84R-298/ 5-29-85	Ethylenethiourea, TD 83-223, lot No. 888-36, 99.8% pure	259044	333 µg/mL	Negative, but insufficient dose range	3	Unacceptable
13. Mutagenicity and DNA-damaging Activity for Several Pesticides Tested with Bacillus subtilis mutants. Mutation Res. 71(1980): 169-179/Texas Tech Univ.-EPA Contract No. 68-01-3963	Dithane (technical) zinc and manganese ethylene-bisdithio-carbamate	N/A--Published data provided by reviewer/ sponsor	(a) 50-300 µg/ plate	(a) Positive DNA damage in B. subtilis at 50 µg/plate--non-activated and 300 µg/plate S9-activated	(a) 3	(a) Acceptable
			(b) 1-25 µg/plate (Liquid incubation using DMSO as solvent)	(b) Positive mutagen in B. subtilis TKJ 6321. Potency = 38.4 mutants/nanomole, nonactivated. Positive mutagen in S. typhimurium TA1535 at 2 µg/plate, nonactivated	(b) 1	(b) Acceptable
14. DNA-damaging and Direct Effects on DNA in Bacillus subtilis/ Thesis, Texas Tech Univ.- EPA Contract CR806904-01; NCI Grant 1-R01-CA21020-02A1/B-80	Dithane (technical) zinc and manganese ethylene-bisdithio-carbamate	N/A--Thesis data provided by reviewer/ sponsor	(a) 50-300 µg/ plate	(a) Positive DNA damage in B. subtilis strain hcr-9, rh2004-1, and mc-1 at all levels, nonactivated	(a) 3	(a) Acceptable
			(b) 10-50 µg/mL (liquid)	(b) Positive at 10 µg/mL in B. subtilis hcr-9 rh2004-1, and mc-1, nonactivated	(b) 3	(b) Acceptable
			(c) 10 µg/mL	(c) DNA in whole cells damaged for gene transformation	(c) 3	(c) Acceptable
			(d) 100 µg/ 100 µg DNA	(d) Purified DNA damaged for gene transformation	(d) 3	(d) Acceptable

TABLE 1. One-Liner Summary Table of Mutagenicity Studies with Mancozeb (Dithane M-45) and Ethylenethiourea (continued)

Study / Lab / Study Date	Material	Accession No.	Dose	Conclusions (Evaluations)	Genetic Effects Category	Classification
15. Mutagenicity--Phage Induct Test for DNA Damage (Escherichia coli strain B13(λ+)B-galactosidase assay)./Thesis, Texas Tech Univ.- EPA Contract CR806904-01/5-81	Dithane (technical) zinc and manganese ethylene-bisdithio-carbamate	M/A-- Thesis data provided by re-viewer/ sponsor	50-300 µg/plate 1-50 µg/mL (liquid assay)	Negative in plate assay Weakly positive in tube assay at 50 µg/mL	3 3	Acceptable Inconclusive, presumptive positive

005425

TOXICOLOGY BRANCH DATA REVIEW

CHEMICAL: Mancozeb

Caswell No.: 913A
EPA Chem No.: 014504

STUDY TYPE: Chronic (2-yr) Toxicity (Feeding) - Rat

CITATION: Toxicological Study on the Effects of Adding Dithane-45 to the Diet of Rats for a Period of 90 Weeks.

ACCESSION No.: N/A

MRID: ~~N/A~~ 080713

SPONSOR: Rohm & Haas Company, Philadelphia, PA

TESTING LAB.: Department of Pharmacology, Medical College of Virginia (J.F. Borzelleca, A.M. Ambrose and P.S. Larson)

STUDY NO.: N/A

DATE: November 9, 1965

Test Material:

Dithane-45, 86% ai (presumably the technical; no other identifiers).

Procedures:

Young (28-day) weanling Wistar albino rats of both sexes (25/sex/group) were fed test material at levels of 0, 25, 50, 100, and 1000 ppm in Purina Lab Chow, and weighed once weekly. From the 12th to the 26th week, treated males and females were mated twice to provide F1's for a three-generation reproduction study (reported separately), then continued for the projected 2-year chronic study. Food consumption, hematologic (hct, hb, CBC) and urinary (presence of reducing substances and protein) values, and metabolic rate (calculated from oxygen consumption) were monitored periodically. At sacrifice, absolute and relative organ weights (heart, spleen, kidneys, liver, testes, adrenal, thyroid) were recorded, and additional tissues (lungs, bladder, gi tract, bone marrow, skin, brain, pituitary, pancreas) subjected to histopathological examination.

Study Result and Conclusions:

The study had to be terminated at 90 weeks (21 months) because of high mortality in all groups. Survival during the in-life portion was summarized as follows (extracted from TABLE 1 of the FINAL REPORT):

Sex	Dietary Conc. (ppm)	Survivors at Month Indicated: 0	6	12	18	21 (term)
Female	0	25	22	20	15	7
	25	25	22	20	15	8
	50	25	20	18	12	7
	100	25	18	17	13	8
	1000	25	24	23	19	14
Male	0	25	24	22	16	11
	25	25	22	19	15	12
	50	25	21	18	11	6
	100	25	22	18	15	8
	1000	25	22	18	12	7

No consistent differences from controls were reported in any test group for body weight, food consumption, hematologic or urinary values, metabolic rate or organ weights. The only significant histological finding noted was thyroid hyperplasia (graded according to the scheme attached to this review) in 1000 ppm males and females, as well as in a few males each at lower levels:

Number of Thyroids Examined (Indicated Grade of Hyperplasia)

Sex	Dose (ppm): 0	25	50	100	1000
Male	12 (0)	9 (0) 3 (1)	7 (0)	6 (0) 4 (1)	5 (0) 3 (1) 2 (2) 1 (3)
Female	8 (0)	11 (0)	12 (0)	8 (0)	6 (0) 6 (1) 5 (2)

Monthly summary progress reports were submitted, but no individual animal data were provided in the FINAL REPORT.

005425

TB Evaluation/Core:

Because of the absence of clinical chemistries and insufficient number of animals examined histopathologically, this 90 week dietary study in rats is classified Core -Supplementary.

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

8-28-86

John C. Harris 8/28/86
Section Head, Section VI

Grading of Thyroid Hyperplasia (copied from page 4 of FINAL REPORT):

Grade 0: Normal range of variation in size of follicles, amount of colloid, staining properties of colloid, height of epithelium, and nuclear characteristics. This embraces Seifter & Ehrich's grades 0 and +, both of which they consider to be within the normal range.

Grade 1: Denotes the presence of numerous microfollicles with thin watery colloid and cuboidal epithelium of increased height. This grade may very occasionally be found as a spontaneous lesion in rats and corresponds to Seifter & Ehrich's grade + +.

Grade 2: Numerous hyperplastic and hypertrophied follicles with scanty, poorly-staining colloid; nuclei are large with increased chromatin content. Such glands are not seen in spontaneous disease or in normal animals and reflect a goitrogenic process. This corresponds to Seifter & Ehrich's grade + + +.

Grade 3 Gland enlarged due to follicular hyperplasia and hypertrophy; colloid scant and absent; cuboidal cells tall, often with vacuoles, and containing large active nuclei; infoldings or papillary processes prominent. This corresponds to Seifter & Ehrich's grades + + + + and + + + + +.

EPA: 68-02-4205
DYNAMAC No. 205-C1
October 1, 1986

005425

DATA EVALUATION RECORD

MANCOZEB

Special Study

STUDY IDENTIFICATION: Larson, P. S., Wodes, W. R., and Ambrose, A. M. Correlative study of functional and morphologic changes in the thyroid glands of rats receiving Dithane M-45 or propylthiouracil in the diet. (Unpublished study prepared by the Medical College of Virginia, Department of Pharmacology, submitted by Rohm and Haas Company, Philadelphia, PA; dated December 30, 1965.) MRID No. 080713.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
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Signature: I. Cecil Felkner

Date: 10-1-86

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1. CHEMICAL: Mancozeb; Dithane M-45.

005425

2. TEST MATERIAL: Dithane M-45.

3. STUDY/ACTION TYPE: Special Study.

4. STUDY IDENTIFICATION: Larson, P. S., Wodes, W. R., and Ambrose, A. M. Correlative study of functional and morphologic changes in the thyroid glands of rats receiving Dithane M-45 or propylthiouracil in the diet. (Unpublished study prepared by the Medical College of Virginia, Department of Pharmacology, submitted by Rohm and Haas Company, Philadelphia, PA; dated December 30, 1965.) MRID No. 080713.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

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Date: Oct. 1, 1986

Margaret Brower, Ph.D.
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Jane Harris, Ph.D.
EPA Section Head

Signature: Jane E. Harris
Date: 10/03/86

7. CONCLUSIONS:

A. When Dithane M-45 was fed to rats for 3 weeks at 1000 ppm in the diet, body weights of females but not males were slightly, but decreased and thyroid-to-body weight ratios were increased in males (significantly) as well as in females (non-significant). The uptake of [^{131}I] was not affected, but protein-bound iodine (PBI) was significantly increased ($p < 0.05$) in both sexes receiving 100 or 300 ppm; however, PBI was significantly decreased ($p \leq 0.05$) in females receiving 1000 ppm when compared to controls.

The metabolic rate was decreased ($p < 0.05$) in males receiving 1000 ppm. Significant hyperplasia (grade 2) was seen in the thyroids of one male and one female that received 1000 ppm Dithane M-45. A NOEL was not achieved; the LOEL was 100 ppm, the lowest dose tested.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. F_{2a} generation Wistar rats from a reproduction study on Dithane M-45 were used in this study. Groups of 15 males and 15 females were continued on diets containing 0, 100, or 1000 ppm Dithane M-45 for 3 months. A group of weanlings from the F_{1b} generation that had been fed 25 ppm in the diet were continued on diets containing 300 ppm. For studies on propylthiouracil (PTU), 10 males and 10 females from the F_{1a} 0-ppm group received 300 ppm PTU in the diet for 3 months and similar groups were continued on 0 ppm for 8 weeks and then received 1333 ppm PTU in the diets for 5 weeks.

2. Metabolic rate measurements were made in month 3. Rats were placed in a wide-mouthed quart jar that was immersed in a water bath at 35°C and connected to a respirometer; this allowed an accurate measurement of oxygen consumption. Carbon dioxide was absorbed in a soda-lime trap. Oxygen consumption was converted to cal/square meter body surface/hour.

3. Twenty-four hours prior to sacrifice, the rats received an intraperitoneal injection of carrier-free Na[^{131}I] (63,000 cpm). After 24 hours, animals were anesthetized and blood drawn (vena cava) for determination of PBI. The thyroid glands were dissected, weighed, and fixed in formalin. [^{131}I] uptake into thyroid was determined.

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[1] Only items appropriate to this OER have been included.

4. Sections of thyroid were examined histologically and graded for hyperplasia.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

Mean body weights of females receiving 1000 ppm Dithane M-45 for 13 weeks were slightly but significantly lower than controls; there was no effect on males. There were large decreases in mean body weights of males and females receiving PTU for 3 months at 300 ppm or from weeks 8-13 at 1333 ppm (Table 1).

Thyroid-to-body weight ratios were significantly ($p < 0.05$) increased in males receiving 1000 ppm Dithane M-45, but only slightly increased (nonsignificant, $p > 0.05$) in 1000-ppm females. Thyroid-to-body weight ratios in both groups of males and females receiving PTU were 3- to 6-fold increased ($p \leq 0.05$) when compared to controls (Table 1).

The uptake of [^{131}I] was not significantly increased ($p > 0.05$) in males or females dosed with Dithane M-45, but significant decreases were found in both sexes dosed with 300 or 1333 ppm PTU. PBI was significantly ($p < 0.05$) increased in both sexes receiving 100 or 300 ppm and was significantly ($p \leq 0.05$) decreased in females receiving 1000 ppm Dithane M-45 when compared to controls. There was no effect on PBI in groups fed PTU (Table 2).

Decreased metabolic rates ($p < 0.05$) were seen in males but not females receiving 1000 ppm Dithane M-45. Metabolic rates were decreased in males and females receiving PTU; the decreases were significant ($p \leq 0.05$) in males receiving 300 and 1333 ppm and females receiving 300 ppm (Table 3).

Significant hyperplasia (grade 2) was seen in the thyroids of one male and one female that received 1000 ppm Dithane M-45. In contrast, all animals receiving PTU had grade 3 thyroid hyperplasia. Data on grading of thyroids are summarized in Table 4.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. There were indications that Dithane M-45 caused thyroid dysfunction in animals receiving 1000 ppm for 13 weeks. Changes in PBI levels, an increase in both sexes receiving 100 and 300 ppm Dithane M-45, and a decrease in females receiving 1000 ppm occurred without changes in [^{131}I] uptake. It was suggested that Dithane M-45 inhibited peripheral deiodination of thyroxine. The lower PBI in females receiving 1000 ppm Dithane M-45 was not interpretable; a decreased synthesis of thyroxine was not supported by a decreased metabolic rate in high-dose females.

TABLE 1. Mean Body Weights and Thyroid-to-Body Weight Ratio (±SD) in Rats Fed Dithane M-45 or Propylthiouracil (PTU)

	Dithane M-45 (ppm)				PTU (ppm)	
	0	100	300	1000	300	1333
Males	(10)[a]	(10)	(10)	(10)	(9)	(7)
Body Weight (g)	402 ±33	384 ±40	386 ±41	407 ±23	237 ±31*	372 ±26*
Thyroid/Body Wt. (mg/kg)	54.1±5.7	46.8±8.3	58.8±9.2	67.2±12.5*	332.1±61.5*	191.6±54.8*
Females	(11)	(10)	(10)	(11)	(10)	(9)
Body Weight (g)	240 ±19	231 ±14	272 ±29	221 ±16*	197 ±34*	229 ±14*
Thyroid/Body Wt. (mg/kg)	68.0±7.6	62.8±9.7	70.8±10.5	76.3±15.4	416 ±1365*	285.0±72.7*

[a]The number of animals examined is in parenthesis.

*Significantly different from control value ($p \leq 0.05$).

TABLE 2. [^{131}I] Uptake and Serum Protein-Bound Iodine (PBI) in Rats Fed Dithane M-45 or Propylthiouracil (PTU)

	Dithane M-45 (ppm)				PTU (ppm)	
	0	100	300	1000	300	1333
Males						
[^{131}I] Uptake (%)	13.01±1.29[a]	15.00±4.13	10.54±2.88	15.7±2.74	4.14±1.83*	1.05±0.36*
PBI (µg %)	2.08±1.17	3.69±1.34*	3.62±0.99*	2.82±1.16	2.18±1.04	2.04±0.45
Females						
[^{131}I] Uptake (%)	12.58±3.28	13.55±5.45	10.47±4.47	13.88±7.78	4.81±3.97*	1.90±0.85*
PBI (µg %)	2.46±0.61	3.88±1.31*	3.23±0.86*	1.62±1.00*	2.28±0.78	2.02±0.61

[a]The values are means ±SD for 7-11 rats/group.

TABLE 3. Metabolic Rate (cal/m^2/hr) in Rats Fed Dithane M-45 or Propylthiouracil (PTU)

	Dithane M-45 (ppm)				PTU (ppm)	
	0	100	300	1000	300	1333
Males	39.2±3.1[a]	36.2±6.8	41.4±2.8	20.1±4.6*	24.2±2.8*	25.4±5.3*
Females	29.8±6.1	28.8±1.3	22.2±5.4	33.0±6.8	21.9±0.9*	25.1±1.0

[a]Values are mean ± SD for five rats.

TABLE 4. Number of Rats with Thyroid Hyperplasia and Severity of Finding after Feeding Dithane M-45 or Propylthiouracil (PTU)

Grade of Hyperplasia[a]	Dithane M-45 (ppm)				PTU (ppm)	
	0	100	300	1000	0	1333
Males						
	(10)[b]	(10)	(10)	(10)	(9)	(7)
0	10	10	10	6	--	--
1	--	--	--	3	--	--
2	--	--	--	1	--	--
3	--	--	--	--	9	7
Ave. grade	0	0	0.5	0.5	3	3
Females						
	(11)	(10)	(10)	(11)	(10)	(9)
0	11	9	8	9	--	--
1	--	1	2	1	--	--
2	--	--	--	1	--	--
3	--	--	--	--	10	9
Ave. grade	0	0.1	0.2	0.3	3	3

[a]Grade 0--Within the normal range.

Grade 1--Numerous microfollicles with watery colloid and cuboidal epithelium of increased height.

Grade 2--Numerous hyperplastic and hypertrophied follicles with scanty poorly stained colloid; nuclei are large and have increased chromatin.

Grade 3--Gland enlarged due to hyperplasia. Colloid absent in follicles, cuboidal cells tall, vacuolated, and with large nuclei; infoldings and papillary processes prominent.

[b]The number of rats examined is given in parenthesis.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study is adequate by 1965 standards. However, the purity of the test material was not stated in this report. It may be the same batch used for the reproduction study (Accession NO. 80713), which had a stated purity of 86%. The amount of thiourea in the preparation was not determined. Individual animal data were present for thyroid parameters and the sampling of mean values we rechecked were accurate. Body weights were only available at study termination. The response of PTU was as expected. More recent work with refined assays indicates that PTU inhibits coupling of iodotyrosine and iodination as well as peripheral conversion of T_4 to T_3. The results indicate that Dithane M-45 produces a mild goitrogenic response when fed to rats at 1000 ppm. The NOEL was not achieved in this study based on increases in PBI at 100 ppm.

Items 15 and 16--see footnote 1.

EPA: 68-02-4225
DYNAMAC No. 205-E
October 1, 1986

DATA EVALUATION RECORD

MANCOZEB

Three-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Larson, P. S., Borzelleca, J. F., and Ambrose, A. M. Three generation reproduction study on rats receiving Dithane M-45 in their diet. (Unpublished study, including letters dated May 25, 1964; June 9, 1964; July 2, 1964; August 18, 1964; January 20, 1965; February 5, 1965; February 8, 1965; March 1, 1965; May 14, 1965; and October 7, 1965, from P. S. Larson to Allen R. Deschere, by the Medical College of Virginia, Dept. of Pharmacology, for Rohm and Haas, Co. Philadelphia, PA; dated November 12, 1965.) MRID No. 080715.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

Date: _____

1. CHEMICAL: Mancozeb; Dithane M-45.

2. TEST MATERIAL: Dithane M-45 was described as a fungicide consisting of a coordination product of zinc ion and manganese ethylene bisdithiocarbamate. Samples averaged 86% active ingredient.

3. STUDY/ACTION TYPE: Three-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Larson, P. S., Borzelleca, J. F., and Ambrose, A. M. Three generation reproduction study on rats receiving Dithane M-45 in their diet. (Unpublished study, including letters dated May 25, 1964; June 9, 1964; July 2, 1964; August 18, 1964; January 20, 1965; February 5, 1965; February 8, 1965; March 1, 1965; May 14, 1965; and October 7, 1965, from P. S. Larson to Allen R. Deschere, by the Medical College of Virginia, Dept. of Pharmacology, for Rohm and Haas, Co., Philadelphia, PA; dated November 12, 1965.) MRID No. 08C715.

5. REVIEWED BY:

Michael Narotsky, B.A.
Principal Reviewer
Dynamac Corporation

Signature: M. Narotsky
Date: OCT 1, 86

Guillermo Millicovsky, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: G Millicovsky
Date: 1 OCT 86

6. APPROVED BY:

[illegible] and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Date: 10-1-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irving Mauer
Date: 10/07/86

Jane Harris, Ph.D.
EPA Section Head

Signature: Jane C Harris
Date: 10/03/86

7. CONCLUSIONS:

A. Based on the available data, the LOEL for parental toxicity of the compound in rats could not be established; no compound effects on parental animals were demonstrated at any dose level.

The NOEL and LOEL for reproductive toxicity were 100 and 1000 ppm, respectively, based on reduced F_1 and F_2 pregnancy rates and F_{3b} litter sizes at the 1000-ppm level.

8. This report did not include the individual data necessary for a comprehensive evaluation of the results presented; the study predated the 1982 USEPA Pesticide Assessment Guidelines, Subdivision F. Therefore, this study is classified Core Supplementary.

Items 8, 9, and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Dithane M-45, with an average purity of 86%, was mixed with Purina Laboratory Chow to produce test diets containing 0 (control), 25, 100, and 1000 ppm of active ingredient. The test material was suspended in 50 mL Mazola oil and added to the diet. Three vessel rinses of 20, 20, and 10 mL oil were also added. An equal volume of oil was mixed with the control diet. Diets were prepared in 6-kg batches every 6 months and refrigerated. The study authors did not report whether chemical analyses of the diet preparations were performed.

2. Animals and Experimental Design: At 28 days of age, 25 male and 25 female Wistar albino rats were individually housed and assigned to each of four groups; these rats were selected to be F_0 parental animals. After receiving their respective diets for 11 weeks, 20 females of each group were paired with a male from the same group to produce F_{1a} litters. Unmated females were paired with a different male every 7 days for up to three successive 7-day periods. After weaning the F_{1a} litters, F_0 rats were rebred to produce F_{1b} litters.

Thirty male and female rats from each group of F_{1b} weanlings were selected to be F_1 parents and continued on their respective diets. At about 105 days of age, 20 animals/sex/group were bred (using the same procedures described for their parents) to produce F_{2a} and F_{2b} litters.

---

[1] Only items appropriate to this OER have been included.

F_{2b} weanlings were selected, maintained, and bred (using the same procedures) to produce F_{3a} and F_{3b} litters.

3. Observations and Measurements: Parental body weights were recorded at the initiation and end of each litter interval. In addition, premating body weights were recorded weekly for the F_1 and F_2 generations.

The number of females that were mated, pregnant, and delivered was recorded for each breeding interval. The number of live and dead pups was recorded on day 1 of lactation. On day 5, litter sizes were recorded and reduced to a maximum of 10 pups. Litter sizes and weights were recorded on day 21.

After the F_{2b} litter interval, F_1 females that had not delivered their F_{2a} and/or F_{2b} litters were bred a third time.

Relative thyroid weights were determined for 10 rats per sex per group for F_{1b}, F_{2b}, and F_{3b} weanlings and F_1 and F_2 parental animals. Heart, lung, liver, kidney, urinary bladder, spleen, stomach, small intestine, large intestine, bone marrow, skeletal muscle, brain, pituitary, thyroid, adrenal, pancreas, and gonad tissues of 10 F_{3b} weanlings per sex per group were examined histologically.

No other information on study procedures was reported.

4: No statistical methods were reported; however, the study authors reported statistical differences between groups for data of relative thyroid weights.

B. Protocol: A study protocol was not included in the report.

12. REPORTED RESULTS:

A. Test Material Analysis: No methods or results of chemical analyses of the test material in the diet preparations were reported.

B. Parental Data: Survival data prior to the breeding of F_0 animals were not presented; however, mortality data were reported for the remainder of the study (Table 1). The total numbers of reported deaths were 7, 16, 11, and 7 in the control and low-, mid-, and high-dose groups, respectively. No necropsy findings were reported.

Compared to their controls, the average relative thyroid weights of 10 high-dose F_2 males and 10 mid-dose F_1 females were significantly increased and decreased, respectively.

No clinical findings or food consumption were reported. Body weight data of F_0 parental animals prior to breeding also were

TABLE 1. Number of Surviving Rats Fed Dithane M-45

Dose Level (ppm)	Week 0	Week 11	Breeding Initiation	'a' Litter Weaning	'b' Litter Weaning
F_0 Males					
0	25	NA[a]	20	20	20
25	25	NA	20	20	20
100	25	NA	20	19	18[b]
1000	25	NA	20	20	20
F_0 Females					
0	25	NA	20	19	19
25	25	NA	20	20	19
100	25	NA	20	17	17
1000	25	NA	20	20	19
F_1 Males					
0	25	25	20	20	20
25	25	25	20	20	19
100	25	25	20	20	19
1000	25	25	20	20	20
F_1 Females					
0	25	24	20	20	19
25	25	25	20	19	18
100	25	25	20	20	20
1000	25	24	20	20	20
F_2 Males					
0	25	25	20	20	20
25	25	20	20	19	18
100	25	23	20	20	19
1000	25	23	20	20	19
F_2 Females					
0	25	24	20	20	18
25	25	22	20	19	18
100	25	23	20	19	19
1000	25	24	20	19	19

[a]NA--not available; data were not reported.

[b]Eighteen males were reported at the beginning of the 'b' breeding interval. The reported data did not account for the reduction from the 19 males when 'a' litters were weaned.

not presented. Compared to their respective controls the mean body weights of F_1 females and F_2 males receiving the test material were, in general, slightly reduced (Tables 2 and 3). No individual data or statistical evaluations were presented.

C. Reproductive and Developmental Data: Pregnancy rates for all groups at both breeding intervals of the F_0 generation were comparable; however, reduced pregnancy rates were evident in the high-dose group of the F_1 and F_2 generations at both intervals (Table 4). Pregnancy rates of F_2 females were lower than normal for the control and all dose groups; nonetheless, high-dose values were reduced when compared to controls. The percentage of pregnant females delivering was comparable in all groups throughout the study.

The study authors reported that 3, 5, 6, and 10 F_1 females in the control and low-, mid-, and high-dose groups, respectively, did not deliver at either or both of the F_{2a} and F_{2b} litter intervals; these animals were rebred a third time. At this rebreeding, one, one, four, and five (33, 20, 67, and 50%) of these respective females were successfully impregnated.

The study authors reported no compound effects on litter size, pup weight at weaning (Table 5), and pup survival (Table 6).

Relative thyroid weights of selected progeny of the mid-dose male and female F_{1b} weanlings were reported to be significantly decreased when compared to their controls. Significant increases were present in the F_{2b} high-dose males and females, F_{2b} mid-dose females, and F_{3b} high-dose males.

Histological examinations of selected F_{3b} weanlings revealed no compound-related findings.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that lower fertility in the F_1 and F_2 generations occurred at 1000 ppm. They stated that there were no effects of the compound on gestation, lactation, viability, pup body weights at weaning, relative thyroid weights, or histological findings of progeny.

B. A quality assurance statement was not included with the study report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Parental Data: We assess that the reported mortality data did not indicate a compound effect; deaths occurred sporadically in a nondose-related pattern.

TABLE 2. Mean Body Weights (g) Prior to Breeding of Rats Fed Dithane M-45

Dose Level (ppm)	Week 0	1	3	5	8	11
F_1 Males						
0	78	122	202	279	356	389
25	73	111	193	272	345	375
100	78	116	204	278	345	379
1000	72	112	193	269	345	385
F_1 Females						
0	73	107	157	193	225	244
25	71	100	151	187	218	233
100	70	99	150	184	212	228
1000	66	96	142	173	210	222
F_2 Males						
0	NA[a]	106	182	260	333	387
25	NA	98	164	239	306	362
100	NA	107	172	242	315	360
1000	NA	99	166	238	314	363
F_2 Females						
0	NA	91	138	175	204	228
25	NA	91	134	172	202	221
100	NA	88	128	160	185	207
1000	NA	88	132	168	198	221

[a]NA--Not available.

TABLE 3. Mean Body Weights (g) Before, Between, and After Litter Intervals of Rats Fed Dithane M-45

Dose Level (ppm)	At Breeding Initiation	Between Litter Intervals	After All 'b' Litters Weaned
F_0 Males			
0	356	441	472
25	341	426	462
100	327	399	436
1000	333	425	463
F_0 Females			
0	224	261	282
25	224	261	286
100	230	262	283
1000	219	252	284
F_1 Males			
0	389	463	488
25	375	444	468
100	379	440	466
1000	385	463	483
F_1 Females			
0	244	281	304
25	233	263	283
100	228	260	283
1000	222	268	283
F_2 Males			
0	387	484	525
25	362	443	473
100	360	450	486
1000	363	449	491
F_2 Females			
0	228	279	290
25	221	269	293
100	207	245	269
1000	221	259	289

TABLE 4. Summary of Reproductive Data of Female Rats Fed Dithane M-45

	Dose Level (ppm)	No. of Females Paired	Females Pregnant		Females Delivered	
			No.	%	No.	%
F_0 Females						
F_{1a} Interval						
	0	20	19	100	19	100
	25	20	18	90	18	100
	100	20	17	94	16	94
	1000	20	19	95	19	100
F_{1b} Interval						
	0	19	19	100	19	100
	25	20	17	85	17	100
	100	17	15	88	15	100
	1000	20	18	90	17	94
F_1 Females						
F_{2a} Interval						
	0	20	18	90	18	100
	25	20	16	80	16	100
	100	20	16	80	16	100
	1000	20	13	65	13	100
F_{2b} Interval						
	0	20	18	90	17	94
	25	19	14	74	14	100
	100	20	16	80	16	100
	1000	20	12	60	12	100
F_2 Females						
F_{3a} Interval						
	0	20	15	75	15	100
	25	20	15	75	15	100
	100	20	13	65	13	100
	1000	20	11	55	11	100
F_{3b} Interval						
	0	20	11	55	11	100
	25	19	15	79	15	100
	100	20	14	70	14	100
	1000	20	8	40	8	100

TABLE 5. Summary of Litter Size and Pup Weight Data of Rats Fed Dithane M-45

Dose Level (ppm)	No. of Litters	Mean No. Live and Dead Pups Day 1	Mean No. Live Pups Day 1	Mean No. Live Pups Day 5	Mean No. Live Pups Day 21	Mean[a] Pup Weight (g) Day 21
F_{1a} Litters						
0	19	10.5	10.0	9.2	8.4	33.5
25	18	10.3	9.8	9.6	8.2	31.3
100	16	10.4	10.4	10.2	9.3	31.9
1000	19	10.4	9.9	9.4	8.6	33.3
F_{1b} Litters						
0	19	9.8	8.9	8.5	6.7	39.1
25	17[b]	12.1	10.6	10.4	7.7	36.3
100	15	12.1	11.4	10.9	8.0	35.2
1000	17	11.5	10.3	9.9	8.1	37.7
F_{2a} Litters						
0	18	9.3	9.1	8.9	7.8	40.5
25	16	11.3	11.2	11.0	8.7	38.9
100	16	10.8	10.7	10.7	8.6	38.4
1000	13	10.6	9.8	9.2	7.8	40.3
F_{2b} Litters						
0	17	7.9	7.9	7.1	5.9	43.6
25	14	10.6	10.6	10.6	8.2[c]	40.0
100	16	8.8	8.6	8.3	6.4	37.3
1000	12	9.4	9.3	8.7	7.3	37.0
F_{3a} Litters						
0	15	10.7	9.9	7.5	6.4	36.3
25	15	9.3	8.3	5.9	5.3	42.9
100	13	9.9	8.8	7.6	6.3	41.3
1000	11	9.5[d]	8.2	6.9	5.2	43.3
F_{3b} Litters						
0	11	9.4	9.2	9.1	7.5	43.9[e]
25	15[b]	10.9	9.6	9.1	7.1	41.5
100	14	11.0	10.9	9.9	8.1	40.7
1000	8[b]	7.3	6.4	5.6	4.6	43.7

[a]Average weight of all pups in group.

[b]Includes one litter with all pups dead at day 1.

[c]Does not include one litter whose dam died 2 weeks postpartum.

[d]Reviewers' calculations indicate 8.8

[e]Reviewers' calculations indicate 44.0.

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TABLE 6. Summary of Pup Survival of Rats Fed Dithane M-45

Dose Level (ppm)	No. of Litters	% Pup Survival per Group		
		Day 1	Days 1-5	Days 5-21
F_{1a} Litters				
0	19	99.5	91.6	98.2
25	18	94.6	98.3	95.5
100	16	99.4	98.2	97.4
1000	19	95.9	94.2	98.2
F_{1b} Litters				
0	19	90.4	95.3	85.2
25	17[a]	87.9	97.8	91.0
100	15	92.4	95.3	88.9
1000	17	89.3	96.0	98.6
F_{2a} Litters				
0	18	98.2	98.2	94.6
25	16	98.9	98.3	92.1
100	16	99.4	100.0	98.6
1000	13	92.0	94.5	98.1
F_{2b} Litters				
0	17	100.0	89.6	98.0
25	14	100.0	99.3	86.2
100	16	97.9	96.4	88.7
1000	12	98.2	93.7	100.0
F_{3a} Litters				
0	15	91.9	75.7	96.0
25	15	89.2	71.0	95.2
100	13	88.4	86.8	94.3
1000	11	92.8	84.4	83.8
F_{3b} Litters				
0	11	98.1	99.0	96.5
25	15[a]	87.8	95.1	93.0
100	14	98.7	91.4	100.0
1000	8[a]	87.9	88.2	84.1

[a]Includes one litter with all pups dead on day 1.

Necropsy findings were not reported; hence, they could not be assessed by the reviewers. Relative thyroid weights of F_1 and F_2 adults revealed no consistent differences between groups. We regard these differences to be incidental.

Mean body weights of dosed F_1 females and F_2 males were, in general, slightly reduced when compared to controls; however, there was inconsistency of the data across generations and between sexes. We therefore do not regard these reductions to be compound related. We reserve any further conclusions on this parameter because there were no individual data or statistical analyses of the data available. We regard the absence of F_0 premating body weight data to be unacceptable because of the particular importance of these data in assessing parental effects of the compound.

Reproductive and Developmental Data: We consider the reduced pregnancy rates for the 1000-ppm F_1 and F_2 dams to be indicative of reproductive toxicity. The increased number of F_1 high-dose females not bearing two litters was a consequence of reduced pregnancy rates in these females. In addition, we regard the litter sizes of F_{3b} high-dose litters to be reduced on day 1 (and throughout lactation) and to reflect a reproductive effect at 1000 ppm. No further evidence of reproductive or developmental toxicity was noted from the data presented.

B. In general, we concur with the study authors' interpretation of the reported reproductive data. Although the study authors did not note a reduction in F_{3b} litter sizes at 1000 ppm, this did not alter the assessment of the reproductive NOEL and LOEL. The study authors made no statements regarding parental effects of the compound.

C. Since this study was conducted from 1963-1965, it was not designed to meet the current standards of testing and reporting prescribed in the 1982 USEPA Pesticide Assessment Guidelines, Subdivision F. However, it should be noted that a complete assessment of the compound's parental, reproductive, and developmental effects was precluded by the absence of the following data:

1. Body weights and mortality data of F_0 parental animals.
2. Clinical observations and necropsy findings of adults and progeny.
3. Food consumption of parental animals.
4. Histological findings of reproductive tissues from the control and high-dose parents and progeny.
5. Precoital time.
6. Gestation lengths.
7. Pup weights prior to weaning.
8. Individual data for all parental and litter parameters.
9. Statistical analyses (and methods).

10. Progeny data (e.g., pup survival, pup weight) analyzed using the litter, rather than the pup, as the experimental unit.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-3.

APPENDIX A

Materials and Methods

Mancozeb TOX Review 005425

Page ____ is not included in this copy.

Pages 277 through 279 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

REVIEWER

1294

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D C 20460

005425

005318

JL 21 1986
JL 20 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTAN

MEMORANDUM

SUBJECT: Mancozeb (NRDC) - Evaluation of Data Submitted Under Accession Nos. 261535, 261536, 261537, 261538, 261539

Caswell No. 913A

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

07-10-86

TO: Arvella Farmer, PM 61
Special Review Branch
Registration Division (TS-767C)

THRU: Jane E. Harris, Ph.D., Head Marion Copley for JEH 7/17/86
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Rohm & Haas Company

Action Requested:

Review and evaluate the following studies submitted in response to Data Call-In (DCI):

1. Mancozeb: Hazard Identification, Evaluation, and Extrapolation to Humans, P.K. Chan, November 7, 1985 (EPA Accession No. 261535).

2. Mancozeb: Three-Month Dietary Toxicity Study in Rats, P.R. Goldman, H.J. Bernacki, and D.L. Quinn, Report Number 85R-167, February 27, 1986 (EPA Accession No. 261536).

3. Three-Month Dietary Toxicity Study with Mancozeb in Dogs, R.E. Cox, Report Number 86RC-7, February 26, 1986 (EPA Accession No. 261537).

4. Mancozeb: Two-Week Inhalation Toxicity Study in Rats, R.C. Baldwin, J.V. Hagan, and J.R. Fisher, Report Number 85R-190, February 27, 1986 (EPA Accession No. 261538).

5. Mancozeb: Subchronic Inhalation Toxicity Study in Rats/13-Week Interim Report, R.C. Baldwin, J.V. Hagan, and J.R. Fisher, Report Number 86R-0003, February 27, 1986 and Mancozeb/ETU: Rat Inhalation Study - Exposure Phase: Analysis of Urine, Blood, and Thyroids, Report Number ABM-477-86, February 27, 1986 (EPA Accession No. 261539).

Toxicology Branch (TB) Conclusions

These data have been screened for adequacy to satisfy data requirements for the Mancozeb Registration Standard (Memorandum: Mauer to Farmer, March 31, 1986). That preliminary screening concluded that these studies represent those necessary for satisfying data requirements for subchronic testing in rat and dog by the dietary route, and in the rat by the inhalation route.

As detailed in the Data Evaluation Records (DER's) and summarized below, compoundrelated effects on the thyroid were thoroughly investigated, including residue analysis of both the parent compound (none detected as CS_2 in any EBDC-treated group), and its principal active derivative, ethylene thiourea (dose-related increase of 4 ppm ETU in 30-ppm mancozeb animals to approximately 25 ppm ETU in 1000-ppm animals).

The following summarizes TB's reviews and evaluation of these studies (detailed DER's are attached):

Study (1) is a company-prepared review (with no primary data) of Mancozeb and ETU, containing background toxicological information, hazard identification and evaluation, and extrapolations to humans; appendices on other degradation products and contaminants (EBIS, EU); pharmacokinetics, exposure estimates and thyroid dysfunction/tumorigenesis interrelationship.

TB Conclusions: Accepted as submitted for information only (No DER prepared).

005318

Study (2): Subchronic dietary - rat

005425

Doses fed: 0, 30, 60, 125, 250, and 1000 ppm Mancozeb; 250 ppm ETU.

Reported effects: None for mancozeb at or below 125 ppm; slight change in hormone levels in females fed 250 ppm; depressed body weight and histological changes in thyroid at 1000 ppm.

TB Conclusions: Core-Minimum Data

NOEL (syst) = 60 ppm (equivalent to 3.5/4.4 mg/kg/da, males/females, respectively)
LEL (syst) = 125 ppm (7.4/9.2 mg/kg/da, males/females, respectively), based on renal tubular degeneration in males.
NOEL/LEL for thyroid effects = 125/250 ppm, based on decreases in serum T4 and TSH in females.

Study (3): Subchronic dietary - dog

Doses fed: 0, 10, 100, 1000, and 5000 ppm

Reported effects: None at or below 100 ppm; depressed feed consumption and body weight at 1000 ppm and higher; reduced survival and thyroid effects at 5000 ppm.

TB Conclusions: Core-Minimum Data

NOEL (syst) = 100 ppm (3 mg/kg/da)
LEL (syst) = 1000 ppm (29 mg/kg/da), based on decreased food consumption and body weight gains; cortical lymphoid depletion in thymus; prostatic hypogenesis.
NOEL/LEL for thyroid effects = 1000/5000 ppm (= 102 and 109 mg/kg/day in males and females, respectively), the LEL producing follicular cell hyperplasia, decreased T3 and T4; hypercholesterolemia and hyperbilirubinemia, decreased food consumption and body weight.

Study (4): Subacute inhalation - rat

(To select doses and compare effects of whole body exposure to those of nose-only for the 13-week inhalation study, 5).

Doses tested: 0, 11, 55, and 258 mg/m^3 (respirable conc.)

Reported effects: For whole-body exposure, the "maximum" NOEL was 11 mg/m^3, and LOEL was 55 mg/m^3 (lower body weight gain and T3 levels in males, lower T4 levels in both sexes). For nose-only exposure, NOEL was 55 mg/m^3 and LOEL was 258 mg/m^3 (lower male body weight gains and T3/T4 levels; histopathological changes in respiratory tract of both sexes).

TB Conclusions: Core-Supplementary Data (range-finding)

NOEL (whole body) = 11 mg/m^3 (respirable)
LEL (whole body) = 55 mg/m^3 (respirable), based on decreased T4 in males and females; decreased T3 in males.

NOEL (nose only) = 55 mg/m^3 (respirable)
LEL (nose only) = 258 mg/m^3 (respirable), based on decreased body weight in males; decreased T4 and T3 in males.

(Nose-only exposure selected for Study 5.)

Study (5): Subchronic inhalation - rat

Doses tested (nose-only): 0, 20, 80, and 320 mg/^{3} (nominal)
0, 8, 36, and 144 mg/m^3 (respirable nose-only aerosol concentration).

Interim sacrifice: One-half of each group at 13 weeks.

Reported effects: None at or below 80 mg/m^3 (nominal); body weight changes and thyroid effects at the HDT, 320 mg/m^3 (nominal).

Terminal sacrifice scheduled following a 13-week period of nontreatment in order to study reversibility with no mancozeb exposure. Results of this recovery phase not yet submitted.

TB Conclusions (for 13-week interim data only):
Core-Supplementary Data, pending review of the final report including recovery phase data.

NOEL = 8 mg/m^3 (respirable)
LEL = 36 mg/m^3 (respirable), based on yellow-brown granular pigment in renal tubules of males and females.

NOEL/LEL for thyroid effects = 36/144 mg/m^3 (respirable), based on decreased T4 in females, accompanied by follicular epithelial hyperplasia.

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005318

EPA: 68-02-4225
DYNAMAC No. 1-0098
May 23, 1986

DATA EVALUATION RECORD

MANCOZEB/ETHYLENETHIOUREA

Three-Month Subchronic Toxicity Study in Rats

STUDY IDENTIFICATION: Goldman, P. R., Bernacki, H. J., and Quinn, D. L. Mancozeb: three-month dietary toxicity study in rats. (Unpublished report No. 85R-167, protocol No. 85P-134, prepared and submitted by the Toxicology Department, Rohm and Haas Company, Springhouse, PA; dated February 27, 1986.) Accession No. 261536.

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Date: 5-27-86

005425

1. CHEMICAL: Mancozeb; Dithane M-45; ethylenethiourea.

2. TEST MATERIALS: Mancozeb technical, lot No. 43339, TD No. 85-15, was described as a yellow powder containing 84 percent of the active ingredients, a coordination product of zinc ion and manganese ethylenebisdithiocarbamate. Ethylenethiourea, lot Matheson Coleman and Bell; product No. IX0010, TD No. 85-55, was described as a white crystal containing 99.8 percent active ingredient.

3. STUDY/ACTION TYPE: Three-month subchronic feeding study in rats.

4. STUDY IDENTIFICATION: Goldman, P. R., Bernacki, H. J., and Quinn, D. L. Mancozeb: three-month dietary toxicity study in rats. (Unpublished report No. 85R-167, protocol No. 85P-134 prepared and submitted by the Toxicology Department, Rohm and Haas Company, Springhouse, PA; dated February 27, 1986.) Accession No. 261536.

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7. CONCLUSIONS:

A. Under the conditions of this study, mancozeb and its metabolite, ethylenethiourea (ETU), were toxic when fed to rats at the highest dose levels of 1000 and 250 ppm, respectively, for 13 weeks. Males and females in both test groups showed decreased body weights and food consumption, decreased MFO activity, decreased serum T4 (thyroxine) and increased TSH (thyroid stimulating hormone) levels, increased absolute and relative liver and thyroid weights, and follicular epithelial hyperplasia of the thyroid. High-dose mancozeb females showed increased absolute and relative spleen weights.

Diffused hypertrophy and follicular epithelial hyperplasia of the thyroid were observed in males and females dosed with 1000 ppm mancozeb or 250 ppm ETU. In addition, one ETU-treated male had a follicular adenoma and multicentric lymphosarcoma of the thyroid. Other major histopathological changes were mostly restricted to the males in the high-dose groups of both test compounds and included centrilobular hepatocellular hypertrophy, and increased amounts of hypertrophied cells in the anterior lobe of the pituitary and in the zona glomerulosa of the adrenal cortex.

The LOEL for systemic subchronic toxicity of mancozeb was 125 ppm, based on histopathologic changes in the kidneys of males and females. No compound-related effects were observed at doses up to and including 60 ppm mancozeb; therefore, the systemic NOEL for mancozeb was 60 ppm (3.5 mg/kg/day in males and 4.4 mg/kg/day in females).

8. Core Classification: Core Minimum.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test compound, mancozeb, lot No. 43339, was described as a yellow powder nominally containing 84 percent of the active ingredient, a coordination product of zinc cation and manganese ethylenebisthiocarbamate (Dithane M-45). The second test compound, ethylenethiourea (ETU), (lot Matheson Coleman and Bell, IX0010), was described as a white crystalline solid containing 99.8 percent active ingredient. Individual test diets were prepared each week by homogenizing the test compounds with the basal diet, Purina Certified Rodent Chow #5002-meal (Ralston Purina Co.). The percent of

---

[1] Only items appropriate to this OER have been included.

active ingredient and homogeneity of mancozeb and ETU were determined in the first batch of diets, and each time the concentrations of these compounds were changed in the test diets. Samples from each test diet were collected at the end of weeks 1, 3, 5, 8, and 12 to determine the stability of the test compounds during the feeding week. Mancozeb and ETU were stored at room temperature.

2. Approximately 4-week-old Crl-CD(SD) rats (Charles River Breeding Laboratories) were acclimated to the animal facility for 2 weeks and randomized according to body weight into a control, five mancozeb, and one ETU dose groups of 14 rats/sex/dietary group.

   Animals were caged individually in an environmentally controlled room. In order to maintain approximately the same level of compound intake (mg/kg/day) throughout the feeding period, dose levels in the diets were gradually increased. During the first 2 weeks of study, rats were fed their group diets containing only 50 percent of the final dose levels of the test compounds (15, 30, 62.5, 125, or 500 ppm of mancozeb, and 125 ppm of ETU). The respective dose levels were increased to 70 percent of the final concentrations (21, 42, 87.5, 175, or 700 ppm of mancozeb, and 175 ppm of ETU) during weeks 3-4 of feeding. Between weeks 5-13, rats were fed at the final dose levels of 30, 60, 125, 250, or 1000 ppm for mancozeb and 250 ppm for ETU.

3. Body weights and food consumption were determined 1 week prior to the initiation of treatment, and weekly thereafter. Compound intakes were calculated weekly.

4. Animals were examined daily for signs of toxicity, external lesions, behavior, posture, gait, irregularities in respiration, body temperature, and color and consistency of excreta. Individual ophthalmoscopic examinations were conducted prior to initiation of study and during the last week of treatment.

5. Hematologic (9 tests) and clinical chemistry (16 tests) determinations were made after 13 weeks (day 92) on blood collected from 10 animals/sex/group that had been fasted overnight. Serum samples prepared from the same blood lots were used for assessment of thyroid function by determining thyroxine (tetraiodothyronine, T4), triiodothyronine (T3), and thyroid stimulating hormone (TSH) levels.

6. Representative liver sections were randomly selected from six rats/sex/group (animals bled for laboratory determinations) and processed into microsomal suspensions to determine mixed function oxidase (MFO) activity using the aniline hydroxylation (AH) and aminopyrine (AP)N-demethylation methods.

7. Prior to necropsy, 24-hour urine samples were collected from four rats/sex/group; blood, thyroids, and liver samples were collected at necropsy. Only blood, urine, and thyroid samples were analyzed for ethylenebisdithiocarbamate (EBDC) and ETU residues. Due to the small sample size, thyroids from two 1000-ppm male and female rats were used for EBDC residue analysis. Thyroids from the remaining 1000-ppm males and females were analyzed for ETU residues. Thyroids from other mancozeb groups were analyzed only for ETU residues. All thyroids from ETU rats were analyzed for ETU residue only.

8. All survivors were necropsied after 13 weeks of treatment. All organs, tissues, and body cavities were examined for gross abnormalities. The adrenals, brain, gonads, heart, kidneys, liver, spleen, and thyroid/parathyroid were removed and weighed. Approximately 40 organ/tissues were examined microscopically.

9. Analysis of variance was used to assess the significance of intergroup differences for clinical chemistry and hematologic parameters, and organ weights. Analysis of covariance was used to assess body weight and food consumption data. The parameters for thyroid function were assessed using analysis of variance, followed by Dunnett's t-test.

B. Protocol: A protocol was provided in the study report and is presented in Appendix A of this review.

12. REPORTED RESULTS:

A. Test Compound and Dietary Analysis: Technical grade mancozeb (Dithane) and ETU used in this study actually contained 88.6 and 97.8 percent of the active ingredients, respectively.

The test compounds in the diets stored at room temperature remained stable over a 7-day feeding period, averaging 98 and 91 percent of the nominal values for mancozeb and ETU, respectively. Homogeneity values for the two compounds averaged 100 and 92-98 percent of the nominal values. Percent conversion of mancozeb to ETU in the fresh and stored feed samples averaged 1.9±0.8 and 6.3±0.9, respectively.

The mean intake of mancozeb and ETU over 13 weeks of dosing was calculated as follows (table on p 11 of the CBI report):

Group	Mancozeb Dose (ppm)	Compound Intake (mg/kg/day) Males	Females
2	30	1.78±0.19	2.20± 0.23
3	60	3.49±0.39	4.38± 0.56
4	125	7.42±0.79	9.24± 1.22
5	250	14.98±1.78	17.82± 2.13
6	1000	57.34±6.04	74.64±11.27
7 (ETU)	250	14.28±1.37	17.81± 2.24

B. Clinical Observations and Mortality: Clinical signs frequently observed in all treatment groups included black crusty material around the eyes, and alopecia. Reportedly, these conditions were of common occurrence in the laboratory rat. Individual ophthalmoscopic examinations revealed no compound-related ocular pathology.

One 125-ppm mancozeb male died during the first week of study. The cause of death was not apparent; however, it was not considered to be compound related.

C. Body Weights: No compound-related changes in body weights of animals fed up to and including 250 ppm mancozeb were observed. The mean body weight of males receiving 1000 ppm mancozeb was significantly lower ($p \leq 0.05$) than controls (3-8 percent) between weeks 3-13 (Table 1). ETU males showed a significant decrease in body weight (3-7 percent, $p \leq 0.05$) at study weeks 2-13. Females in the 1000-ppm mancozeb group showed a decrease (3-14 percent) in mean body weight between weeks 2-13; however, differences were significant ($p \leq 0.05$) only between weeks 7-10. The decrease in mean body weight of ETU females ranged between 6-8 percent during the 13-week period; however, the change was statistically significant only at week 2.

D. Food Consumption: No compound-related changes in food consumption were found in groups fed up to and including 250 ppm mancozeb. In general, food consumption in 1000-ppm mancozeb males was significantly depressed (8-15 percent, $p \leq 0.05$) between study weeks 3-13 (Table 2). Food consumption in 1000-ppm mancozeb females was only slightly decreased (1-6 percent) during this period. A significant ($p \leq 0.05$) decrease (8-12 percent) in food consumption in ETU males was observed at study weeks 3-13. ETU females showed a moderate decrease in food consumption (6-10 percent); the change was significant ($p \leq 0.05$) at weeks 1, 3, and 6. At four instances during the study, significant increases (7-9 percent, $p \leq 0.05$) in food consumption were observed at Mancozeb levels of 30-250 ppp. Reportedly, such sporadic increases were not compound related.

TABLE 1. Summary of Mean Body Weight (g±SD) in Rats Fed Mancozeb[a,b] or ETU[b]

Test Compound (ppm)	Week 0	2	3	4	6	8	10	12	13
MALES									
Control	140.9 ±10.1	245.5 ±19.2	297.5 ±24.0	338.8 ±29.0	394.5 ±36.4	429.1 ±41.5	460.8 ±47.9	481.8 ±51.5	487.1 ±58.2
Mancozeb (1000)	141.7 ±9.4	242.5 ±18.6	288.7* ±22.3	318.3* ±32.9	364.8* ±49.3	396.8* ±47.5	429.2* ±49.5	446.2* ±49.6	446.7* ±55.0
ETU (250)	140.9 ±9.7	237.6* ±20.7	287.1* ±26.5	321.6* ±30.5	372.8* ±40.5	405.0* ±42.9	433.7* ±50.5	455.5* ±54.6	464.8 ±57.8
FEMALES									
Control	113.6 ±5.9	164.0 ±14.2	185.4 ±18.0	203.2 ±21.8	231.6 ±26.0	247.8 ±29.5	262.2 ±29.8	272.8 ±32.5	278.4 ±34.5
Mancozeb (1000)	114.5 ±5.5	159.9 ±12.3	181.1 ±16.1	196.8 ±20.3	213.6 ±23.0	213.4* ±25.2	239.3* ±25.0	251.6 ±24.1	258.4 ±24.9
ETU (250)	112.1 ±5.7	153.7* ±9.9	172.4 ±13.1	190.6 ±13.0	215.4 ±18.1	228.6 ±19.5	241.3 ±20.2	249.8 ±19.9	257.1 ±20.3

*Significantly different from control value ($p \leq 0.05$).

[a]Only values from the highest dose groups shown; mean body weights from all lower dose groups were comparable to controls.

[b]Mean values based on 14 rats/sex/group.

TABLE 2. Summary of Mean Food Consumption (g±SD) in Rats Fed Mancozeb[a,b] or ETU[b]

Test Compound (ppm)	Week 0	2	3	4	6	8	10	12	13
					MALES				
Control	17.51 ±1.30	23.40 ±2.21	26.62 ±2.59	27.14 ±2.44	27.04 ±2.50	26.36 ±2.28	25.87 ±2.85	26.86 ±3.33	26.33 ±3.92
Mancozeb (1000)	17.58 ±1.54	23.32 ±2.24	24.61* ±2.16	24.23* ±3.77	24.02* ±3.79	22.50* ±5.80	24.23 ±3.06	23.69* ±2.98	23.36* ±4.21
ETU (250)	18.76 ±1.96	23.58 ±2.09	24.62* ±2.65	25.08* ±2.46	23.85* ±2.78	23.27* ±2.39	23.45* ±3.26	24.37* ±2.94	24.34* ±3.50
					FEMALES				
Control	15.59 ±1.30	17.54 ±1.63	18.39 ±1.89	18.98 ±2.13	19.50 ±1.95	19.03 ±1.95	19.13 ±1.80	19.26 ±1.79	19.50 ±2.38
Mancozeb (1000)	16.18 ±1.36	17.21 ±1.89	17.57 ±2.01	18.49 ±2.27	18.39 ±1.88	19.51 ±3.76	18.30 ±2.26	18.48 ±1.67	19.23 ±1.89
ETU (250)	15.30 ±1.10	16.51 ±1.28	16.62* ±1.53	17.81 ±1.51	17.47* ±1.54	17.55 ±1.51	17.31 ±1.27	18.23 ±1.36	18.11 ±1.36

*Significantly different from control value ($p \leq 0.05$).

[a]Only values from the highest dose groups shown; mean food consumption from all lower dose groups were comparable to controls.

[b]Mean values based on 14 rats/sex/group.

Intake of mancozeb and ETU (mg/kg/day) was calculated from the nominal dose levels, mean body weights, and food consumption. In general, compound intake in females, especially at the highest dose level, was higher than in males. Compound intake at all dose levels in both sexes was increased at week 5 and leveled off to a fairly consistent value by week 13.

E. Hematology: Hematological parameters exhibiting significant ($p \leq 0.05$) differences included increased (3 percent) mean corpuscular hemoglobin concentration (MCHC) in 125-ppm mancozeb males, decreased (19 percent) platelets in ETU males, increased white blood cells (WBC) in 60- and 125-ppm mancozeb males (42-43 percent), and decreased monocytes in 1000-ppm mancozeb (82 percent) and ETU (95 percent) males. Reportedly, all of these differences were considered random and not compound related. No significant changes were observed in females.

F. Clinical Chemistry: There were significant ~~compound-related~~ changes in clinical chemistry parameters ~~were~~ restricted to the high-dose groups. Males receiving 1000 ppm mancozeb showed significant ($p \leq 0.05$) increases in blood urea nitrogen (84 percent), creatinine (28 percent), and cholesterol (52 percent). Females in the same dose group showed increased serum alkaline phosphatase (32 percent) and triglyceride (90 percent) levels compared to controls. However, each of these increases resulted primarily from exceptionally high values obtained for male No. 3627 and female No. 3858; therefore, group increases were not considered compound related. Serum cholesterol levels were significantly increased in ETU males (69 percent) and females (30 percent). All statistically significant changes in clinical chemistry parameters in low- and mid-dose mancozeb females were sporadic, and not considered compound related.

G. Thyroid Function: Serum T4 levels were significantly decreased ($p \leq 0.05$) in 1000-ppm mancozeb males (34 percent) and 250- (28 percent) and 1000-ppm (43 percent) mancozeb females (Table 3). Serum TSH levels were significantly increased in males (26 percent, $p \leq 0.05$) and females (169 percent) receiving 1000 ppm mancozeb. In ETU rats, serum T4 levels were significantly decreased ($p \leq 0.05$) in males (50 percent) and females (65 percent). T3 levels were significantly increased ($p \leq 0.05$) in males (28 percent) and females (16 percent), and TSH levels were significantly increased (408 percent in males, 263 percent in females).

H. Hepatic Mixed Function Oxidase: Mancozeb at the highest dose level nonsignificantly decreased the MFO activity in males (31 percent) and females (40 percent) when measured by aniline hydroxylation. MFO activity was significantly reduced by 32 percent ($p \leq 0.05$) in ETU males when measured by aminopyrine N-demethylation. A decrease in MFO activity due to mancozeb or ETU treatment was evident irrespective of the basis (per mg microsomal protein, per g liver, or per total liver) for activity determination.

TABLE 3. Mean Serum Levels (±SD) of Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH) in Rats Fed Mancozeb or ETU for 13 Weeks[a]

Test Compound (ppm)	Males			Females		
	T3 (ng/mL)	T4 (µg/dL)	TSH (ng/mL)	T3 (ng/mL)	T4 (µg/dL)	TSH (ng/L)
Control	1.22±0.17	5.27±0.98	1.20±0.58	1.40±0.17	3.78±1.08	0.49±0.23
Mancozeb (30)	1.19±0.16	5.32±0.65	1.13±0.48	1.44±0.16	3.39±0.49	0.68±0.34
Mancozeb (60)	1.16±0.21	5.35±1.21	1.75±1.50	1.42±0.16	3.55±0.62	0.66±0.37
Mancozeb (125)	1.30±0.15	5.65±0.85	1.58±0.94	1.35±0.13	3.20±0.41	0.39±0.34
Mancozeb (250)	1.28±0.14	5.28±0.80	1.88±1.21	1.37±0.15	2.71*±0.40	0.95±0.70
Mancozeb (1000)	1.31±0.19	3.49*±1.05	4.33*±2.53	1.35±0.19	2.16*±0.62	1.32*±1.02
ETU (250)	1.56*±0.26	2.62*±0.72	6.10*±3.18	1.63*±0.30	1.34*±0.47	1.78*±1.14

*Significantly different from control value ($p \leq 0.05$).

[a]Means based on serum samples prepared from 10 animals/sex/group, except for TSH in female control group, where only nine determinations were made.

I. Residue Analysis: Urine, blood, and thyroid samples were analyzed for presence of EBDC and ETU. No EBDC or ETU residues were detected in the blood samples drawn from the mancozeb-treated animals. The amount of ETU in urine samples from mancozeb-treated animals increased in a dose-related manner from approximately 0.3 ppm at 30-ppm mancozeb to 10 ppm at the 1000-ppm mancozeb dietary concentration. The urine samples from rats fed 125 to 1000-ppm mancozeb also contained 0.10 to 1.1 ppm of EBDC residues. The average total amount of ETU excreted in the urine in 24 hours is summarized in Table 4.

No EBDC residues above the detection limit of 25 ppm were detected in thyroids obtained from the 1000-ppm mancozeb rats. Analysis of thyroid samples of mancozeb-treated animals showed a dose-related increase in ETU residue; values ranged from less than the detection limit of 4 ppm in 30-ppm mancozeb animals to approximately 25 ppm in the 1000-ppm animals.

ETU levels in the blood of ETU-treated animals were found to be marginally above the detection limit of 0.1 ppm; levels ranged from 2.9 to 63 ppm in the urine and from 30 to 53 ppm in the thyroids.

J. Organ Weights: Compound-related changes in organ weights were restricted to the highest dose groups. Relative liver weights were significantly increased ($p \leq 0.05$) in 1000-ppm mancozeb males (11 percent) and females (24 percent) and ETU males (12 percent) and females (15 percent) (Table 5). Mean absolute and relative weights of thyroids were significantly ($p \leq 0.05$) increased in mancozeb (32 and 49 percent) and ETU males (80 and 84 percent); in mancozeb and ETU females relative thyroid weights (33 and 80 percent, respectively) were significantly ($p \leq 0.05$) increased. Relative spleen weights were also significantly ($p \leq 0.05$) increased in 1000-ppm mancozeb females (22 percent) and ETU females (16 percent).

Because of increased body weights, absolute liver, heart, and kidney weights in 60-ppm mancozeb males were significantly ($p \leq 0.05$) increased. In addition, a few random but statistically significant changes in organ weights in 30-, 250- and 1000-ppm mancozeb animals were observed.

K. Gross Pathology: Three ETU-treated males showed enlarged livers. Prominent lobular architecture and pale or discolored livers, observed in many of the treatment groups, were not considered compound related.

L. Histopathology: The major compound-related histopathological changes were restricted to the liver, kidneys, thyroid, adrenal, and pituitary glands (Table 6). Thyroids in 1000-ppm mancozeb and ETU rats showed a diffused hyperplasia of the follicular epithelium. In the thyroid of one 1000-ppm mancozeb and three

TABLE 4. Mean Levels of ETU (µg±SD) Excreted in 24-Hour Urine of Rats Fed Mancozeb or ETU for 13 Weeks[a]

	Mancozeb (ppm)						ETU (ppm)
	0	30	60	125	250	1000	250
Males	<0.030	1.35 ±1.28	3.99 ±2.46	14.91 ±6.17	18.45 ±12.88	97.29 ±31.47	52.58 ±28.64
Females	<0.010	0.93 ±0.51	2.43 ±0.81	5.3 ±1.14	18.09 ±10.03	83.07 ±29.80	98.30 ±92.46

[a]Means based on 4 animals/sex/group.

TABLE 5. Mean Absolute (g) and Relative Organ Weights (Organ Wt. x 10,000/Body Wt.) of Rats Fed Mancozeb[a,b] or ETU for 13 Weeks[b]

Test Compound (ppm)	Males						Females					
	Liver		Spleen		Thyroid		Liver		Spleen		Thyroid	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Control	12.29	271	0.667	14.9	0.025	0.551	6.52	256	0.390	15.5	0.019	0.755
	2.58[c]	21	0.135	2.4	0.004	0.070	0.70	24	0.049	2.9	0.004	0.105
Mancozeb (1000)	12.51	300*	0.694	17.1	0.033*	0.822*	7.56	317*	0.447	18.9*	0.024	1.005*
	2.11	35	0.100	5.1	0.010	0.425	1.76	44	0.071	2.8	0.002	0.091
ETU (250)	13.55	303*	0.589	13.0	0.045*	1.013*	6.97	295*	0.426	18.0*	0.032	1.358*
	2.05	32	0.101	1.9	0.014	0.245	0.60	22	0.079	2.6	0.004	0.189

*Significantly different from control value ($p \leq 0.05$).

[a]Only values for the highest dose groups shown.

[b]Means based on 10 animals/sex/group, except for thyroid and spleen in male ETU group, where organs from 9 animals were weighed.

[c]Standard deviation.

TABLE 6. Summary of Histopathological Observations in Rats Fed Mancozeb or ETU for 13 Weeks

Organ/Lesion[a]	Males							Females						
	Dose Group[c]							Dose Group[c]						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Adrenal Glands	10[b]	10	10	10	10	10	10	10	10	10	10	10	10	10
Cortical vacuolation	3	3	2	5	6	1	4	0	0	0	0	0	0	0
Hypertrophy, zona glomerulosa	1	0	1	2	2	6	6	1	1	1	3	1	3	2
Kidneys	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Yellow-brown pigment, cortical tubules														
—minimal	0	0	0	2	4	4	0	0	0	0	3	4	1	0
—slight	0	0	0	7	4	5	0	0	0	0	1	4	4	0
—moderate	0	0	0	0	2	1	0	0	0	0	0	2	5	0
Multifocal cortical tubular degeneration	2	2	5	8	4	4	2	0	1	0	0	1	2	0
Hyaline material, cortical tubules	6	5	6	7	6	4	2	0	0	0	0	0	0	0
Liver	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Hypertrophy, centrilobular hepatocytes														
—minimal	0	0	0	0	0	2	4	0	0	0	0	0	0	1
—slight	0	0	0	0	0	0	2	0	0	0	0	0	0	0
—moderate	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Multifocal mononuclear cellular infiltration	8	9	7	7	9	9	5	5	5	4	2	5	2	3
Pituitary	10	10	9	10	10	10	10	10	10	10	10	10	10	9
Hypertrophied/ vacuolated cells														
—minimal amount	5	8	5	5	6	3	0	6	2	3	3	4	4	1
—small amount	4	2	4	3	3	1	2	0	1	1	2	0	1	4
—moderate amount	1	0	0	1	1	4	4	0	0	0	0	0	0	1
—marked amount	0	0	0	0	0	2	3	0	0	0	0	0	0	0
Spleen	10	0	0	0	0	10	10	10	0	0	0	0	10	10
Hemosiderosis														
—minimal	1	0	0	0	0	1	1	0	0	0	0	0	0	0
—slight	3	0	0	0	0	1	1	0	0	0	0	0	0	2
—moderate	5	0	0	0	0	3	2	3	0	0	0	0	2	3
—marked	1	0	0	0	0	0	0	7	0	0	0	0	8	5
Lymphosarcoma	0	0	0	0	0	0	1	0	0	0	0	0	0	0

[a]Selected from Appendix 14 of the CBI report as recording compound-related changes/lesion.

[b]Numbers of organs examined are underlined.

[c]Dose groups: 1, control; 2-6, 30, 60, 125, 250, and 1000 ppm mancozeb; 7, 250 ppm ETU.

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Continued TABLE 6. Summary of Histopathological Observations in Rats Fed Mancozeb or ETU for 13 Weeks

Organ/Lesion	Males							Females						
	Dose Group							Dose Group						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Thyroid	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Hyperplasia, follicular epithelium	0	0	0	0	0	9	10	0	0	0	0	0	9	10
Basophilic focus/foci	0	0	0	0	0	1	3	0	0	0	0	0	0	0
Cystic follicle(s)	0	0	1	0	1	1	2	0	0	0	0	1	0	0
Ultimobronchial bodies	1	3	3	3	1	1	1	1	2	2	2	1	1	4
Ultimobronchial bodies, cystic	2	2	1	3	1	3	4	3	1	0	0	3	3	2

ETU males, there was a small, well-defined basophilic focus of hyperplastic follicular epithelial cells. One ETU male had follicular adenoma and a multicentric lymphosarcoma in the thyroid.

All treatment groups including controls contained large hypertrophied vacuolated cells of the pituitary; however, the number of these cells was increased in the 1000-ppm mancozeb males. There was an apparent increase in severity (moderate to marked) in males receiving the highest dose levels of mancozeb and ETU.

The kidneys from males and females fed 125 to 1000-ppm mancozeb had minimal to moderate amounts of a yellow-brown pigment in the lumen of the cortical tubules. Pigmentation was reportedly due to deposits of ethylenebisisothiocyanate (EBIS), a yellow-colored excretory metabolite of mancozeb; no histopathological changes were associated with this pigmentation.

An increased incidence of hypertrophy of cells of the zona glomerulosa of the adrenal cortex occurred in the 1000-ppm mancozeb and ETU males; incidence was low in the other treatment groups. Cortical vacuolization of the adrenal gland was found in all male dose groups.

Two 1000-ppm mancozeb males, four ETU males, and one ETU female showed hypertrophy of the centrilobular hepatocytes. Livers from all males and females in groups, including controls, showed a high incidence of multifocal mononuclear infiltration.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that:

1. No compound-related clinical signs or mortalities due to mancozeb or ETU feeding were observed.

2. Food consumption and body weights for both sexes at the highest dose levels of mancozeb and ETU were reduced.

3. None of the mancozeb groups showed any significant compound-related changes in hematologic or clinical chemistry parameters. ETU animals showed increased levels of serum cholesterol; ETU males showed significant decreases in platelet counts.

4. Serum T4 levels were decreased in 1000-ppm mancozeb males and 250 to 1000-ppm mancozeb females. TSH levels were increased in the 250-ppm mancozeb females and 1000-ppm mancozeb males and females.

   ETU males and females showed decreased serum T4 and increased T3 and TSH levels.

5. Hepatic mixed function oxidase activity was reduced in the 1000-ppm mancozeb animals and ETU males.

6. Mean absolute and relative liver and thyroid weights were increased in the high-dose animals for both the test compounds; absolute and relative weights for spleen were increased in 1000-ppm mancozeb females.

7. Deposits of yellow-brown granular pigments were seen in the lumen of the cortical tubules of the kidney in rats at 125 to 1000 ppm mancozeb; however, this condition was not considered a manifestation of compound-related toxicity.

8. Follicular epithelial hyperplasia of the thyroid was observed in high-dose mancozeb and ETU animals; males in both dose groups showed increased amounts of hypertrophied cells in the anterior lobe of the pituitary, and hypertrophy of the cells of zona glomerulosa of the adrenal cortex. Centrilobular hepatocellular hypertrophy was observed in all ETU rats and 1000-ppm mancozeb males.

9. The NOEL for mancozeb was established at 125 ppm and the LOEL was 250 ppm based on decreases in serum T4 and TSH levels in females.

10. No EBDC or ETU residues were detected in the blood samples of mancozeb animals; a dose-related increase was found in the amount of ETU in the urine of mancozeb-treated animals. EBDC residues of 0.1 to 1.1 ppm were also detected in rats fed 125 to 1000 ppm mancozeb. No EBDC residues above the detection limit of 25 ppm were detected in thyroids from high-dose mancozeb rats. ETU residues in thyroids of mancozeb-treated animals increased in a dose-related manner. ETU levels in the blood of ETU-fed animals were found to be slightly above the detection limit of 0.1 ppm; levels in urine ranged from 2.9 to 63 ppm and levels in thyroids ranged from 30 to 53 ppm.

8. A quality assurance statement was signed and dated February 12, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The experimental design was complete and adequate for assessment of the systemic subchronic toxicity of mancozeb and its metabolite (ETU). The summary data tables were supported by individual animal data. The report is well organized and technically sound. Under the conditions of the study, compound-related effects were restricted to the 1000-ppm mancozeb and 250-ppm ETU (highest doses tested) animals, to a major extent in the males. These effects in males and females of both groups included decreased body weights and food consumption, decreased MFO activity, decreased serum T4 and increased TSH levels.

and increased absolute and relative liver and thyroid weights. Follicular epithelial hyperplasia of the thyroid was observed in both sexes of the 1000-ppm mancozeb and ETU animals. High-dose mancozeb females showed increased absolute and relative spleen weights.

Histopathological changes that specifically occurred in the males of 1000-ppm mancozeb and 250-ppm ETU groups were centrilobular hepatocellular hypertrophy, increased amount of hypertrophied cells in the anterior lobe of the pituitary and in the zona glomerulosa of the adrenal cortex, and basophilic foci of hyperplastic follicular epithelium. In addition, follicular adenoma and multicentric lymphosarcoma of the thyroid were found in one ETU male.

The compound-related lesions in the mancozeb-fed rats were histologically similar to those that occurred in the same tissues of the ETU-fed rats.

Many nonspecific clinical conditions, including black crusty material around the eyes and alopecia, were frequently observed in all the treatment groups.

There were no toxicologically important effects on mortality or ocular pathology. One death (125-ppm mancozeb male), though it remained unexplained, was not considered compound related.

Although discounted by the authors as being compound-related, the deposits of yellow-brown pigment present in the lumen of renal cortical tubules (at all doses above 60 ppm) were accompanied by an increased incidence of multifocal cortical tubular degeneration in 125 ppm mancozeb males. These inclusions may represent a form of kidney urolithiasis as seen with gout, cystinuria, or hyperoxaluria. Therefore, these renal inclusions cannot be ignored.

Based on histopathological changes in the kidneys at levels of 125 ppm and above, we assess that a systemic NOEL for mancozeb is 60 ppm (3.5 mg/kg/day in males and 4.4 mg/kg/day in females).

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 48-60.

APPENDIX A

Protocol

Mancozeb TX Review 005425

Page ____ is not included in this copy.

Pages 304 through 316 are not included.

The material not included contains the following type of information:

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DYNAMAC No. 1-009-C
May 23, 1986

005425

DATA EVALUATION RECORD

MANCOZEB

Subchronic Oral Toxicity Study in Dogs

STUDY IDENTIFICATION: Cox, R. H. Mancozeb: Three-month dietary toxicity study in dogs. (Unpublished study No. 86RC-7 prepared by Hazleton Laboratories America, Vienna, VA, for Rohm and Haas Co., Spring House, PA, and E.I. du Pont de Nemours and Co., Wilmington, DE; dated February 26, 1986.) Accession No. 261537.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 10-22-86

1. CHEMICAL: Mancozeb; Dithane M-45; Manzate 200; coordination product of zinc ion and manganese ethylenebisdithiocarbamate; $C_4H_6N_2S_4MnZn$.

2. TEST MATERIAL: Mancozeb (lot No. 43339; TD No. 85-15) was described as a yellow powder containing 83.35 percent active ingredient.

3. STUDY/ACTION TYPE: Subchronic oral toxicity study in dogs

4. STUDY IDENTIFICATION: Cox, R. H. Mancozeb: Three-month dietary toxicity study in dogs. (Unpublished study No. 86RC-1 prepared by Hazleton Laboratories America, Vienna, VA, for Rohm and Haas Co., Spring House, PA, and E.I. du Pont de Nemours and Co., Wilmington, DE; dated February 26, 1986.) Accession No. 261537.

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7. CONCLUSIONS:

Under the conditions of this study, dose levels of 1000 and 5000 ppm of mancozeb in the diet of dogs induced anorexia, loss of body weight, and pale mucous membranes. The dogs in the high-dose group (5000 ppm) had a reduction in red cell mass with associated changes in hematological parameters (decreased hematocrit and hemoglobin levels and decreased erythrocyte counts), as well as increased total bilirubin and cholesterol values and decreased T3 and T4 (thyroid functions) values. Histopathologic examination revealed hypothyroidism, thymic hypoplasia, and hypoplasia of the gonads and sex organs in the mid- (1000 ppm) and high-dose (5000 ppm) animals. Three dogs (two males and one female) in the high-dose group were sacrificed in extremis because of a deterioration in physical condition due to malnutrition. Based on the results of this study, the NOEL for subchronic toxicity is 100 ppm and the LOEL is 1000 ppm for both male and female dogs.

Core Classification: The study is Core Minimum.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, mancozeb (Dithane M-45) from lot No. 4339, was received in the laboratory in two shipments. It was described as a yellow powder containing 83.35 percent active ingredient. All dose calculations in this study were adjusted to 100 percent active ingredient. Mancozeb is insoluble in water and decomposes at 100°C in the presence of moisture; however, it is stable at room temperature under normal light. The details of the chemical profile of the compound are on file with the registrant. Mancozeb was stored refrigerated in a desiccator during the study.

2. The test animals were beagle dogs obtained from Hazleton Research Products, Inc. (Denver, PA). The dogs were quarantined and acclimatized in the laboratory for at least 20 days. Following quarantine, 60 healthy dogs (30 males and 30 females) were selected and randomly assigned to five dose groups, each containing 6 animals/sex. Groups 1-5 received 0, 10, 100, 1000, or 5000 ppm of mancozeb in the diet, respectively. The dogs were 25 to 30 weeks old and weighed between 6.5 and 9.6 kg (males) and 5.2 and 8.2 kg (females) at study initiation. The dogs were housed individually (in cages) in an environmentally controlled room with a 12-hour light/dark cycle. Purina Certified Canine Diet No. 5007,

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[1] Only items appropriate to this OER have been included.

containing the specific dose levels of the test material, was provided to each dog for 2 hours a day through the early part of week 5 and 6 hours each day thereafter except on the days prior to blood collection for clinical laboratory evaluations. Approximately 400 g of feed was offered to each dog through week 3; thereafter, it was reduced to 300 g/day. Water was available ad libitum.

3. The diets were prepared by premixing a weighed amount of the test material with approximately 1 kg of dog feed. The premix was then added to the appropriate amount of feed for each group and mixed in a Patterson-Kelly blender. Fresh diets were prepared once each week. Samples from each group were collected once prior to study initiation and from the 7- and 14-week mixings and were analyzed for homogeneity. Stability of the test material in the diet was determined for samples collected at each mixing during the study including pretest and following 7-day storage at room temperature .

4. The dogs were observed twice daily for mortality and overt toxic effects. A detailed physical examination was performed on all dogs once a week for the first 4 weeks and biweekly thereafter. Ophthalmoscopic examinations were performed on all dogs, once prior to study initiation and again during week 13. Individual food consumption was recorded daily, and body weights were recorded weekly throughout the study. Blood samples were collected from all dogs during weeks -3, -2, 5, and 13 for hematology (10 parameters) and clinical chemistry (16 parameters). Blood collected during weeks 5 and 13 was also used to determine serum concentrations of T3 and T4 (RIA) for evaluation of thyroid function. Levels of TSH were not determined because of the stated unavailability of reliable RIA assay kits.

5. After week 13 of the study, all surviving dogs were sacrificed by exsanguination following sodium thiamylal anesthesia. Necropsies were performed on all dogs, and all gross findings were appropriately recorded.

   Terminal body weights and the weights of the following organs of each animal were recorded: adrenals, brain, testes with epididymides, ovaries, thyroid/parathyroid, kidneys, liver/gallbladder, spleen, and heart. The organ/body and organ/brain weight ratios were calculated from these values.

   Representative portions of 36 organs and tissues of all dogs, in addition to any gross lesions, were fixed in 10% neutral buffered formalin, processed, and examined microscopically after staining with hematoxylin and eosin.

6. Prior to necropsy, 24-hour urine samples were collected from four males and four females randomly selected from each

group. Blood samples were also taken from the same animals. These samples, along with one lobe of the thyroid of all surviving animals, were frozen immediately and sent to Enviro-Bio-Tech (Bernville, PA) for analysis of ethylenebisdithiocarbamate (EBDC) and ethylenethiourea (ETU) residues. Two liver samples were also taken from the same animals from which urine and blood were collected; these samples were frozen and saved for possible future analysis.

7. Body weights, body weight gains, food consumption, hematology, clinical chemistry, and organ weight data were analyzed using appropriate statistical methods. A difference in mean values between dose groups and controls was considered significant at the $p \leq 0.05$ level.

8. Protocol: The study protocol is presented in Appendix A.

12. REPORTED RESULTS:

A. Diet Analysis and Compound Intake: The results of the diet analyses confirmed the homogeneity of the diet preparation. The test material was found to be stable in the diet during the assay period. The average concentration of mancozeb was 106±6.7 percent of the target values for the various diet preparations.

The mean intake of mancozeb over the 13 weeks of dosing was calculated for each group as shown in Table 1.

TABLE 1. Mean Mancozeb Intake[a]

Group	Dose (ppm)	Compound Intake (mg/kg/day ± SD) Males	Females
1	0	0	0
2	10	0.29± 0.025	0.32± 0.024
3	100	2.98± 0.021	3.35± 0.189
4	1000	28.62± 2.315	28.91± 3.016
5	5000	101.53±13.342	108.67±19.152

[a]Calculated from 13 weekly values of mean body weight and mean food consumption.

B. Mortality: Two males and one female in the 5000-ppm dose group were sacrificed in extremis due to a deterioration in physical condition which was caused by anorexia and malnutrition; these findings were reported to be compound related.

C. Clinical Observations: Dose-related clinical signs of dehydration, thinness, and pale mucous membranes were noted in animals of both sexes in the 5000-ppm dose groups, and occasional instances of dehydration were seen in animals in the 1000-ppm dose group (Table 2). These signs were considered the result of malnutrition due to anorexia caused by dosing with the test material.

Physical examination of the animals revealed thinness, dehydration, and pale mucous membrane in high-dose groups as noted in the clinical observations. These were considered to be compound related. Ophthalmoscopic examination of the dogs did not reveal any compound-related effects.

TABLE 2. Incidence of Selected Clinical Findings in Dogs Fed Mancozeb for 3 Months

	Male Dose Group (ppm)					Female Dose Group (ppm)				
	0	10	100	1000	5000	0	10	100	1000	5000
Dehydration	1	0	0	4	6	0	0	1	2	5
Few or no feces	2	1	0	1	6	0	2	1	2	5
Languid	0	0	0	0	1	0	0	0	0	1
Thin	1	0	0	0	2	0	0	0	0	5
Pale mucous membranes	0	0	0	0	2	0	0	0	0	2
Sacrificed in extremis	0	0	0	0	2	0	0	0	0	1

E. Food Consumption and Body Weight: Anorexia occurred as a compound-related effect in animals of both sexes in 1000- and 5000-ppm dose groups beginning at week 1 and continuing throughout the study. The decrease in food intake was approximately 10-20 percent in the 1000-ppm dose group and amounted to a decrease of approximately 40 percent in the 5000-ppm dose group (Table 3).

The decrease in food intake by animals in the 5000- and 1000-ppm groups affected body weight. Males and females in the 5000-ppm dose group lost an average body weight of 0.8 and 1.5 kg, respectively, whereas the control males and females gained an average of 1.1 and 1.0 kg of respectively, over the 13-week study period (Table 4). Body weight gains of both males and females fed 1000 ppm were less than one-half of the control values.

TABLE 3. Selected Mean Food Consumption in Dogs Fed Mancozeb for 3 Months

Dose Level (ppm)	Group Mean Food Consumption (g/week) ±S.D. at Week 0	4	8	13	Total Food Consumption (Weeks 1-13)
			Males[a]		
0	1595.7±421.45	1869.3±126.92	1914.8±175.37	1886.5±162.74	23946.2±1586.8
10	2047.7±265.03	1898.3±110.31	1930.0±169.04	1911.3±202.50	25641.3±1947.8
100	1775.0±232.26	1953.7± 84.61	1940.2± 97.76	1896.3±172.83	25036.2±1179.4
1000	1243.5±202.14	1650.8±261.73	1830.7±118.33	1651.3± 99.70	21504.0±1634.1*
5000	688.8±133.51*	1099.8±141.38*	1020.5±364.78*	1031.3±192.73*	14419.5±1541.1*
			Females[b]		
0	1525.3±194.20	1628.7±216.60	1516.8±137.83	1450.0±314.66	20268.2±2259.3
10	1653.5±219.74	1772.3±115.05	1573.7±130.43	1478.5±132.49	21372.3±1213.1
100	1649.3±232.68	1640.8±313.12	1784.5±216.18*	1597.2±240.41	21967.0±2375.9
1000	952.7±158.30*	1358.5±113.74	1343.0± 86.39	1230.3±202.57	16480.3± 954.5*
5000	601.0±309.38*	924.3±196.42*	1031.6±141.28*	977.8±177.86*	11662.2±1035.7*

[a] Mean values based on 6 animals/group except for the 5000-ppm males at week 13, which included 4 animals.

[b] Mean values based on 6 animals/group except for the 5000-ppm females at weeks 8 and 13, which included 5 animals.

* Significantly different from control value ($p \leq 0.05$).

TABLE 4. Selected Mean Body Weights in Dogs Fed Mancozeb for 3 Months

Dose Level (ppm)	Group Mean Body Weight (kg) ±S.D. at Week 0	4	8	13	Mean Body Weight Change (from wk 0-13)
			Males[a]		
0	8.3±1.03	8.5±0.92	9.1±0.91	9.4±0.93	1.1±0.42
10	8.8±0.40	9.4±4.51	9.8±0.74	10.0±0.81	1.2±0.56
100	8.2±0.91	8.9±0.99	9.4±1.19	9.8±1.17	1.7±0.71
1000	8.0±0.65	8.0±0.74	8.4±0.82	8.5±0.87	0.5±0.55
5000	8.0±1.03	7.1±0.86*	7.0±1.25*	7.4±0.91*	-0.8±0.30*
			Females[b]		
0	6.3±0.44	6.8±0.34	7.1±0.44	7.3±0.64	1.0±0.50
10	6.8±0.48	7.2±0.34	7.5±0.25	7.5±0.28	0.7±0.25
100	6.4±0.54	6.7±0.49	7.5±0.86	7.7±1.04	1.3±0.72
1000	6.3±0.90	6.1±0.78	6.3±0.77	6.2±0.66	-0.1±0.50*
5000	6.8±1.37	5.6±1.28	6.0±0.79*	5.6±0.67*	-1.5±0.65*

[a] Mean values based on 5 animals/group except for the 5000-ppm males at week 13, which included 4 animals.

[b] Mean values based on 5 animals/group except for the 5000-ppm females at weeks 8 and 13, which included 5 animals.

* Significantly different from control value ($p \leq 0.05$).

F. Clinical Pathology (hematology, clinical chemistry, and thyroid function tests): Decreased erythrocyte counts, and hematocrit and hemoglobin levels (Table 5) as well as decreased T3 and T4 values and increased total bilirubin and cholesterol values (Table 6) were observed in animals of both sexes in 5000- and 1000-ppm dose groups. These findings were considered to be due to compound-related hypothyroidism. Decreased alanine aminotransferase (ALT) and calcium values noted in these animals were also considered to have the same etiology. However, the authors also considered the possibility that anorexia and body weight loss may have caused the abnormal clinicopathological findings in the 1000- and 5000-ppm dose groups. No compound-related ophthalmologic changes were reported.

G. Organ Weights: Significantly increased mean absolute and relative thyroid/parathyroid weights were seen in the 5000-ppm males and females and were considered to be the direct result of dosing with mancozeb (Table 7). Other changes in organ weights noted in this study were considered to be due to body weight losses resulting from anorexia.

H. Gross Observations: Enlarged and/or dark thyroid/parathyroids and decreased thymus size were seen in the 1000- and 5000-ppm males and females and were considered compound related. A pale appearance of the visceral organs was noted in two males and one female in the 5000-ppm group which were sacrificed in extremis. No other findings were considered treatment related.

I. Histopathology: Compound-related histomorphological tissue alterations include thyroid follicular cell hyperplasia in both males and females in the 5000-ppm dose group, thymic cortical lymphoid depletion in the 1000- and 5000-ppm animals (both sexes), hypoplastic changes in the reproductive systems of males and females in the high-dose group as well as prostatic hypogenesis in males receiving 1000 ppm, pallor of the zona fasciculata of the adrenal gland in high-dose males and females, and hematopoietic alterations in the spleen and liver of high-dose (5000 ppm) males and females. Normal staining variability in the adrenal cortex was also noted in animals from all groups, including controls. No other compound-related histopathologic alterations were noted. Table 8 summarizes selected microscopic observations.

J. Residue Analysis: The analyses were performed by the gas chromatographic method. ETU and EBDC (as CS_2) were detected in the urine in a dose-dependent manner. Blood levels of ETU were slightly above the detection limit, which is 0.040 ppm, in animals in the 5000-ppm dose group. EBDC was not detected in thyroid; however, ETU was detected in the thyroids of animals of both sexes. The average ETU concentration in the thyroids was 7.78 and 13.02 ppm for males and 5.35 and 10.66 ppm for females in 1000 and 5000 ppm dose groups, respectively (Table 9).

TABLE 5. Selected Mean (± S.D.) Hematology Values of Dogs Fed Mancozeb for 3 Months

Dose Level (ppm)	HGB (g/dl)		HCT (%)		MCV(fl)		RBC (10^6/µl)		MCH (pg)	
Week:	5	13	5	13	5	13	5	13	5	13
Males										
0	14.5 ±1.11	15.3 ±1.12	40.2 ±3.97	43.1 ±3.02	65.3 ±2.18	65.5 ±2.57	6.15 ±.662	6.59 ±.554	23.5 ±0.85	23.2 ±0.83
10	13.9 ±0.59	14.7 ±0.84	38.8 ±1.76	42.2 ±2.20	66.4* ±1.54	66.3 ±1.65	5.84 ±.248	6.35 ±.262	23.8 ±0.73	23.2 ±0.67
100	14.4 ±0.78	15.2 ±1.62	40.2 ±2.67	43.1 ±4.74	64.8 ±2.24	65.5 ±1.77	6.22 ±.577	6.59 ±.799	23.3 ±0.98	23.1 ±0.83
1000	14.0 ±0.73	14.2 ±1.06	39.3 ±2.46	40.1 ±3.28	67.0 ±2.40	67.6 ±1.87	5.86 ±.413	5.94 ±.547	24.0 ±0.92	23.9 ±0.68
5000	12.2* ±1.82	11.5 ±5.72	34.1* ±4.89	32.9 ±15.58	67.5 ±3.58	72.6* ±5.63	5.06* ±.752	4.66 ±2.411	24.1 ±1.54	25.2* ±0.95
Females										
0	15.3 ±1.89	15.5 ±1.40	42.9 ±5.52	43.9 ±3.89	65.5 ±0.51	65.9 ±1.01	6.55 ±.807	6.66 ±.539	23.3 ±0.33	23.3 ±0.36
10	14.3 ±0.85	14.4 ±0.88	40.2 ±2.85	40.9 ±2.40	65.5 ±0.97	65.7 ±1.61	6.13 ±.424	6.21 ±.358	23.4 ±0.45	23.2 ±0.50
100	14.9 ±0.94	14.1 ±1.26	41.9 ±2.98	40.3 ±3.49	66.4 ±0.98	67.1 ±1.21	6.31 ±.504	6.01 ±.520	23.6 ±0.62	23.6 ±0.30
1000	13.0* ±1.02	13.9 ±1.59	36.1* ±3.31	39.4 ±4.10	67.3 ±2.22	68.0 ±1.87	5.36* ±.540	5.81 ±.724	24.3 ±0.96	24.0 ±0.85
5000	12.0* ±1.69	11.9* ±2.55	33.3* ±4.83	33.8* ±6.65	67.9* ±1.07	70.8* ±4.03	4.91* ±.769	4.81* ±1.128	24.4* ±0.88	24.7* ±0.83

*Significantly different from control value ($p \leq 0.05$).

TABLE 6. Selected Mean (± S.D.) Clinical Chemistry and Thyroid Function Values of Dogs Fed Mancozeb for 3 Months

Dose Level	TBILI (mg/dl)		Calcium (mg/dl)		TCHOL (mg/dl)		ALT (μ/L)		T_3(ng/dl)		T_4 (μg/dl)	
(ppm) Week:	5	13	5	13	5	13	5	13	5	13	5	13
Males												
0	0.1 ±0.00	0.1 ±0.08	10.6 ±0.08	10.7 ±0.31	187 ±19.5	179 ±25.9	25 ±2.6	23 ±5.0	1.2 ±0.27	1.0 ±0.14	2.3 ±1.06	1.8 ±0.54
10	0.1 ±0.04	0.1 ±0.04	10.6 ±0.31	10.6 ±0.35	186 ±25.8	169 ±18.5	32 ±7.0	100 ±21.7	1.0 ±0.08	1.0 ±0.12	1.5 ±0.49	1.2 ±0.38
100	0.1 ±0.04	0.1 ±0.00	10.7 ±0.18	10.4 ±0.44	174 ±24.5	152 ±20.5	27 ±5.2	75 ±15.5	1.0 ±0.15	0.9 ±0.14	1.9 ±0.65	1.4 ±0.64
1000	0.3* ±0.17	0.1 ±0.05	10.7 ±0.12	10.6 ±0.19	208 ±37.1	208 ±43.7	21 ±8.9	20 ±6.5	0.9 ±0.21	1.0 ±0.15	1.4 ±0.50	1.4 ±0.31
5000	0.2 ±0.12	0.2 ±0.10	10.1 ±0.68	10.0 ±0.78	335* ±72.8	383* ±53.1	15* ±5.1	13* ±3.6	0.5* ±0.22	0.9 ±0.45	0.3* ±0.25	0.5* ±0.47
Females												
0	0.1 ±0.08	0.1 ±0.05	10.8 ±0.29	10.8 ±0.53	159 ±23.9	174 ±26.6	24 ±3.2	21 ±1.4	1.0 ±0.13	1.0 ±0.19	2.1 ±0.51	2.1 ±0.62
10	0.1 ±0.05	0.1 ±0.05	10.5 ±0.43	10.4 ±0.40	160 ±31.6	139 ±25.8	20 ±3.1	19 ±2.1	1.1 ±0.15	1.0 ±0.10	2.3 ±0.90	1.8 ±0.41
100	0.2 ±0.08	0.1 ±0.05	10.7 ±0.37	10.3 ±0.35	178 ±24.7	174 ±27.1	21 ±4.6	19 ±3.4	1.1 ±0.26	1.0 ±0.10	1.8 ±0.76	1.6 ±0.48
1000	0.2 ±0.05	0.1 ±0.05	10.4 ±0.50	10.5 ±0.36	229* ±52.2	245* ±55.7	21 ±1.3	21 ±2.4	1.1 ±0.14	1.2 ±0.10	2.0 ±0.46	2.3 ±0.18
5000	0.3* ±0.05	0.2* ±0.08	10.1* ±0.33	10.1 ±0.33	254* ±87.6	289* ±62.8	14* ±2.2	13* ±3.4	0.6* ±0.19	0.9 ±0.22	0.4* ±0.24	0.8* ±0.18

*Significantly different from control value ($p \leq 0.05$).

TABLE 7: Mean Absolute and Relative[a] Thyroid/Parathyroid Weights (± S.D.) of Dogs Fed Mancozeb for 3 Months

Sex/Parameter	Dose Level (ppm) 0	10	100	1000	5000
Males					
-Absolute organ weight (g)	0.98 ±0.24[b]	0.85 0.06	0.98 ±0.22	1.02 ±0.19	2.23* ±0.87
-Organ weight relative to final body weight (%)	0.010 ±0.003	0.008 ±0.001	0.010 ±0.002	0.012 ±0.003	0.031* ±0.014
-Organ weight relative to brain weight (%)	0.013 ±0.004	0.010 ±0.001	0.013 ±0.003	0.014 ±0.003	0.031* ±0.011
Females					
-Absolute organ weight (g)	0.80 ±0.19	0.80 ±0.23	0.77 ±0.22	0.77 ±0.12	1.73* ±0.94
-Organ weight relative to final body weight (%)	0.011 ±0.003	0.011 ±0.003	0.010 ±0.002	0.012 ±0.001	0.031* ±0.017
-Organ weight relative to brain weight (%)	0.011 ±0.003	0.011 ±0.003	0.010 ±0.003	0.011 ±0.002	0.025* ±0.013

[a] Organ weight relative to final body weight and organ weight relative to brain weight.

[b] Standard deviation.

* Significantly different from control value ($p \leq 0.05$).

TABLE 8. Incidence of Selected Microscopic Observations in Dogs Fed Mancozeb for Three Months

Organ/Observation	Sex/Dose Level (ppm)									
	Males					Females				
	0	10	100	1000	5000	0	10	100	1000	5000
Thyroid	(6)[a]	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
-follicular cell hyperplasia	0	0	0	0	6	0	0	0	0	6
Thymus	(6)	(6)	(6)	(6)	(5)	(6)	(6)	(6)	(6)	(5)
-lymphoid depletion, cortex	0	0	0	6	5	0	0	0	6	5
Testes	(6)	(6)	6)	(6)	(6)	-	-	-	-	-
-aspermatogenesis	0	0		0	3	-	-	-	-	-
-hypospermatogenesis	0	0	0	0	2	-	-	-	-	-
-hypogenesis	0	0	0	0	1	-	-	-	-	-
Epididymides	(6)	(6)	(6)	(6)	(6)	-	-	-	-	-
-hypogenesis	0	0	0	0	2	-	-	-	-	-
Prostate	(6)	(6)	(6)	(6)	(6)	-	-	-	-	-
-hypogenesis	0	0	0	2	6	-	-	-	-	-
Ovaries	-	-	-	-	-	(6)	(6)	(6)	(6)	(6)
-hypogenesis	-	-	-	-	-	0	0	0	0	4
Uterus	-	-	-	-	-	(6)	(6)	(6)	(6)	(6)
-hypogenesis	-	-	-	-	-	0	0	0	0	4
Liver	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
-extramedullary hematopoiesis, increased	0	0	0	0	2	0	0	0	0	2
-sinusoidal cell pigmentation	0	0	0	0	4	0	0	0	0	2
Adrenals	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
-pallor, zona fasciculata	0	0	0	0	4	0	0	0	0	3

[a]Number in parenthesis is the number of tissues examined.

TABLE 9. Selected Mean and Range of Ethylenethiourea (ETU) Values Observed in the Blood and Thyroid Glands of the Dogs Fed Mancozeb for 3 Months

Specimen	Sex	Dose Group (ppm)	ETU (Mean) (ppm)	ETU (Range) (ppm)
Blood	F	100	ND*	--
	M	100	ND	<0.040-0.070
	F	1000	0.053	<0.040-0.070
	M	1000	0.043	<0.040-0.050
	F	5000	0.15	0.14-0.19
	M	5000	0.14	0.10-1.9
Thyroid	F	100	0.716	0.45-0.90
	M	100	1.83	0.57-4.0
	F	1000	5.35	4.1-9.2
	M	1000	7.78	4.1-9.9
	F	5000	10.66	7.0-15.0
	M	5000	13.02	6.1-18.0

*Not detectable; limit of detection = 0.040 ppm.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Dietary administration of mancozeb (Dithane M-45) to beagle dogs at dose levels of 1000 and 5000 ppm resulted in significant toxic effects. A compound-related decrease in the triiodothyronine and thyroxin thyroid hormones (T3 and T4) was seen in 5000-ppm dose groups. Hypercholesterolemia and hyperbilirubinemia accompanied hypothyroidism. A moderate to marked decrease in food consumption in dogs in the 5000-ppm dose group resulted in loss of body weight and dehydration. Dogs in the 1000-ppm dose group did not gain weight during the study and occasionally appeared dehydrated. The authors concluded that the toxicological significance of some of the changes in clinical laboratory values and the microscopic alterations that were seen in the gonads, thymus, adrenals, and possibly in some of the hematopoietic tissues may have been confounded by malnutrition.

Based on the results of the study, the NOEL for subchronic toxicity of mancozeb in the dog was considered to be 100 ppm in the diet.

B. A quality assurance statement was dated January 29, 1986 for the toxicity study; a quality assurance statement was dated November 26, 1985 for the residue analyses report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Three dogs, two male and one female, in the 5000-ppm dose group were sacrificed in moribund conditions caused by anorexia. Decreases in body weights and food consumption were noted both in the 1000- and 5000-ppm groups of both sexes. The animals in the high-dose group were thin, dehydrated, and had pale mucous membranes. Reductions of red cell mass and related values were also noted in the high-dose group. In addition, the histomorphological evidence of hypothyroidism, pallor in zona fasciculata of the adrenals, and hypoplasia of the gonads and sex organs were present in the high-dose group in dogs of both sexes, including the animals which were sacrificed in extremis.

Evidence of the concentration of the metabolic product (ETU) of mancozeb in the thyroid relative to blood was noted in this study. In the absence of a control study using ETU, it appears that mancozeb (or its metabolite) directly affected the thyroid and possibly the other endocrine glands (adrenal and gonads). Hypothyroidism is known to affect the hematologic parameters adversely and to cause increased cholesterol levels, as noted in the high-dose animals in this study. However, anorexia (due to unpalatable food) and reduced food consumption, noted in 1000 and 5000 ppm dose groups, most likely caused the loss of body weight. These effects could have also conceivably caused the reduction in hematologic values.

Thyroid stimulating hormone (TSH) analyses, although important criteria to consider when the thyroid is a primary target organ, were not performed in this study due to the unavailability of suitable radioimmunoassay kits. In the final analysis, it appears that both anorexia and hypothyroidism acted synergistically to precipitate the toxic effects of mancozeb in the dogs of the high dose groups.

In summary, the NOEL in this study is 100 ppm which is equivalent to intakes of 3.0 and 3.4 mg/kg/day in males and females, respectively. The LOEL is 1000 ppm which is equivalent to 29 mg/kg/day in both sexes. At this dose there was decreased food consumption and body weight gains, transient reduction in RBC mass (counts, hematocrit, and hemoglobin value), cortical lymphoid depletion in the thymus, and prostatic hypogenesis. Compound-related thyroid changes only occurred at the HDT, 5000 ppm (102 and 109 mg/kg/day in males and females, respectively).

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol, CBI pp. 98-114.

APPENDIX A

Study Protocol

Mancozeb TH Review 00342S

Page ____ is not included in this copy.

Pages 334 through 350 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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005318 005425

EPA: 68-02-4225
DYNAMAC No. 1-0090
May 27, 1986

DATA EVALUATION RECORD

MANCOZEB

Two-Week Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Two-week inhalation toxicity study in rats. (Unpublished study No. 85R-190 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261538.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 5-27-86

005318
005425

1. CHEMICAL: Mancozeb; Dithane M-45; Manzate 200; coordination product of zinc ion and manganese ethylenebisdithiocarbamate; $C_4H_6N_2S_4MnZn$.

2. TEST MATERIAL: Mancozeb (lot No. 43339; TO No. 85-015; product code 6-2804) was described as a yellow powder containing 83.35% active ingredient.

3. STUDY/ACTION TYPE: Two-week inhalation toxicity study in rats.

4. STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Two-week inhalation toxicity study in rats. (Unpublished study No. 85R-190 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261538.

5. REVIEWED BY:

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William L. McLellan, Ph.D.
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Date: 5-27-86

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Date: 5-27-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature:
Date: 6-6-86

Jane Harris, Ph.D.
EPA Section Head

Signature:
Date:

7. CONCLUSIONS:

The purpose of this 2-week inhalation study was to select exposure levels and the mode of exposure for a 90-day inhalation study on mancozeb in rats. Four groups of 12 male (weighing 121-180 g) and 12 female (weighing 98-135 g) Crl:CD(SD)BR rats were exposed to dust aerosols of mancozeb for 6 hours per day for 10 days. Respirable concentrations were 0, 11, 55, or 258 mg/m^3 with mass median diameters of 3.5 to 4.9 µm and geometric standard deviations between 2.2 and 2.5. The four groups were further subdivided into whole-body exposed rats and nose-only exposed rats. Considerable difficulty was experienced in generating the dust aerosols because the daily mean concentrations varied from 5 to 82 mg/m^3 in the low-exposure group, 61 to 300 mg/m^3 in the mid-exposure group, and 402 to 681 mg/m^3 in the high-exposure group. These daily concentrations yielded total mean aerosol concentrations of 0, 23, 138, and 519 mg/m^3; however, the respirable fraction ranged from 38 to 49%, so that the mean respirable aerosol concentrations were 0, 11, 55, and 258 mg/m^3, respectively.

Whole-Body Exposure: No deaths occurred during this study. Significant reductions were found in body weight and weight gain for rats exposed to 258 mg/m^3, as well as significant reductions in female body weight and male and female weight gain in rats exposed to 55 mg/m^3. No exposure-related effects were noted in rats exposed to 11 mg/m^3. Male and female thyroxine (T_4) levels and male 3,5,3'-triiodothyronine (T_3) levels were significantly decreased after exposure to 55 or 258 mg/m^3 mancozeb for 2 weeks. Thyroid-stimulating hormone (TSH) levels were nonsignificantly increased in rats exposed to 258 mg/m^3 mancozeb. In addition, male and female lung weights and lung-to-body weight ratios were significantly increased in rats exposed to this same concentration. Exposure-related multifocal interstitial inflammation, microgranulomas, multifocal mixed inflammatory cell infiltration, focal or multifocal necrosis in the respiratory tract, and reactive lymphoid hyperplasia of the peribronchial lymph nodes were also found at 258 mg/m^3.

Nose-Only Exposure: One male exposed to 258 mg/m^3 died during this study due to asphyxiation caused by the animal twisting its head away from the nasal opening in the exposure tube. Alopecia was noted around the eyes of one female exposed to 55 mg/m^3. No adverse effects were noted in males or females exposed to 11 or 55 mg/m^3. Significant reductions in mean body weight (week 2) and mean body weight gain were noted during weeks 1 and 2 in males exposed to 258 mg/m^3. There were no body weight effects in females exposed to 258 mg/m^3. In addition, the T_3 and T_4 levels were significantly reduced and the lung-to-body weight ratio was increased in males exposed to 258 mg/m^3. Microscopic examination of nasal turbinates revealed an increased incidence and degree of multifocal mixed inflammatory cell infiltration, i.e., mononuclear cells and neutrophils and multifocal or focal necrosis of the turbinate mucosa in four males and two females exposed to 258 mg/m^3.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

A. The study was conducted in a reasonable manner, and a signed quality assurance statement dated February 17, 1986, was included.

B. Based on these results, nose-only was selected as the mode of exposure for the 90-day study, and based on the nose-only exposures, 258 mg/m^3 is the LOEL and 55 mg/m^3 is the NOEL for rats exposed to mancozeb for 10 exposures.

C. Whole-body exposed animals were exposed to mancozeb dermally through fur and skin aerosol impaction and orally through preening (during and postexposure) and normal lung clearance mechanisms in addition to exposure via chamber air inhalation. As a result, it was assessed that these animals received a greater concentration of mancozeb than the nose-only exposed rats, as evidenced by the lower LOEL and NOEL, 55 mg/m^3 and 11 mg/m^3, respectively.

D. This 2-week study was not designed as a Core study, but provides useful information. There were deficiencies in the generation system.

9. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-4.

APPENDIX A

Materials and Methods

Mancozeb TR Review 005425

Page ____ is not included in this copy.

Pages 356 through 359 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

005318

EPA: 68-02-4225
DYNAMAC No. 1-009E
June 4, 1986

00542

DATA EVALUATION RECORD

MANCOZEB

Subchronic Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Subchronic inhalation study in rats--thirteen-week interim report. (Unpublished study No. 86R-003 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261539.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 6-9-88

369

1. CHEMICAL: Mancozeb; dithane M-45®; Manzate 200®; coordination product of zinc ion and manganese ethylenebisdithiocarbamate; $C_4H_6N_2S_4MnZn$.

2. TEST MATERIAL: Mancozeb (lot No. 4339; TD No. 85-015; product code 6-2804) was described as a yellow powder containing 83.35 percent active ingredient.

3. STUDY/ACTION TYPE: Subchronic inhalation toxicity study in rats.

4. STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Subchronic inhalation study in rats--thirteen-week interim report. (Unpublished study No. 86R-003 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261539.

5. REVIEWED BY:

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Subchronic Toxicology
Technical Quality Control
Dynamac Corporation

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Date: 6/4/86

Irving Mauer, Ph.D.
EPA Reviewer

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Date:

Jane Harris, Ph.D.
EPA Section Head

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Date:

7. CONCLUSIONS:

A. Groups of 38 male and 38 female rats were exposed to mancozeb target concentrations of 0, 20, 80, or 320 mg/m^3 for 13 weeks. Groups of five males and five females were sacrificed after 4 weeks of exposure, and groups of 16 males and 16 females were necropsied at the end of the 13 week exposure. In addition, 17 males and 17 females were held for an additional 13 weeks following the exposure phase of the study as a recovery study. [The results of the recovery study, were not included in this report.]. The actual mean respirable concentrations to which the rats were exposed were 0, 8, 36, and 144 mg/m^3, respectively. The male rats exposed to 144 mg/m^3 exhibited significant ($p \leq 0.05$) reduced mean body weight and body weight gain for most of the exposure period, whereas no such effects were noted in female rats. Mean corpuscular volume, mean corpuscular hemoglobin concentration, serum triglyceride levels, and inorganic phosphorus levels were significantly ($p \leq 0.05$) altered; however, they were within normal ranges for rats and were not considered of biological relevance. Thyroid function tests revealed significantly reduced T4 serum levels in female rats exposed to 144 mg/m^3 for 13 weeks. In samples collected at the termination of exposures, blood, urine, and thyroid samples exhibited an exposure-response increase in ethylenethiourea (ETU) and ethylenebisdithiocarbamate (EBDC) concentrations. These data support the hypothesis that mancozeb is metabolized to ethylene-thiourea. Organ weight changes included reduced kidneys and heart weights in male rats exposed to 144 mg/m^3; this may have reflected the reduced body weight of these animals. No remarkable ophthalmologic findings were reported. Among the histologic findings, hyperplasia of the follicular epithelium was noted in 3 of 10 females exposed to 144 mg/m^3, yellow-brown granular pigment in kidneys of both males and females exposed to 36 or 144 mg/m^3, and several lesions in the respiratory tract. The thyroid changes were related to exposure to mancozeb whereas the respiratory tract lesions are typically observed following exposure to dusts. The respiratory tract lesions may be indicative of a progressive disease. The authors considered renal inclusions to represent the elimination of a urinary metabolite. However, the granular form of the pigmented material may be indicative of progressive disease or lead to chronic lesions following the inhalation of mancozeb. Based on the renal inclusions, the LOAEL is 36 mg/m^3 and the NOAEL is 8 mg/m^3 for rats exposed to mancozeb dust aerosols via nose-only exposure.

Item 8--see footnote 1.

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[1] Only items appropriate to this OER have been included.

9. BACKGROUND: The exposure levels selected for this subchronic study were derived from a 2-week inhalation study designed to determine exposure levels and the mode of exposure. Nose-only was selected over whole body exposure. From the 2-week study, the NOAEL was 55 mg/m^3 and the LOAEL was 258 mg/m^3 for rats exposed to mancozeb for 10 exposures based on the respirable concentrations of mancozeb in the chambers.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, mancozeb, contained 83.35 percent active ingredient, which was a coordination product of zinc ion and manganese ethylenebisdithiocarbamate. The exposure concentrations used in this study were based on the formulated test material, as received. Four groups of animals, designated 1, 2, 3, and 4, were exposed to target aerosol concentrations of mancozeb of 0, 20, 80, or 320 mg/m^3. Animals were exposed (nose-only) 6 hours a day, 5 days a week.

2. Thirty-eight male and 38 female Crl:CS(SD)BR rats (Charles River-Lakeview, Newfield, NJ) were randomly assigned to each of the four exposure groups. The rats were 35 days old at the initiation of the study and weighed between 157 and 214 g (males) and 124 and 168 g (females). Due to an error in sexing, group 2 contained 37 males and 39 females. The animals in each of the four groups were further divided into three subgroups, designated A, B, and C. Animals from subgroup A were necropsied after 4 weeks, and subgroup B animals were necropsied after 13 weeks of exposure. Subgroup C animals were to be necropsied after a 13-week post-exposure recovery period; data for the recovery period will appear in the final report.

3. The animals were housed individually in stainless steel wire-mesh cages in an environmentally controlled room while they were not in the exposure chambers. The room was maintained at a temperature ranging between 70 and 80°F (21-27°C), a relative humidity of 30-70 percent, and a 12 hour light, 12 hour dark cycle. During exposure, the exposure chambers were maintained under similar conditions. Animals were provided food and water ad libitum except during exposure and during the pre-necropsy fasting period. During exposure, animals were housed in individual PVC nose-only restraining tubes which were attached to the front of the exposure chambers.

4. The powdered test material was placed in a sample reservoir, and was forced through the funnel-shaped bottom of the reservoir by a plunger, into a conducting duct which contained a blow-jet nozzle. From the conducting duct, the test material was incorporated into an air stream which entered the air-mixing turret of a stainless steel and glass exposure chamber. The configuration and stroke frequency of the plunger were varied in order to achieve the different exposure concentrations. The exposure chambers were supplied with filtered room air, and the chamber air flow rate was monitored. The aerosol concentrations in each exposure chamber were also monitored periodically each day in order to check and adjust the aerosol output of the dust generators. The actual chamber analytical concentrations and the particle size distributions were determined gravimetrically. Samples were taken daily from all four chambers to determine chamber analytical concentrations. Particle size samples were taken weekly from chambers 2, 3, and 4, which housed groups 2, 3, and 4, respectively; particle size samples were not taken from chamber 1 (control chamber). The exposure concentrations cited in the report were the respirable dust concentrations, which were calculated from the total analytical dust concentrations and the respirable fraction. The respirable fraction was calculated from the mass median diameter and the geometric standard deviation. Temperature and humidity were monitored continuously in the chambers and in the animal holding room.

5. All animals were examined and weighed the day before the first exposure (week 0), and then weekly until study termination. Animals were examined before, during, and after each exposure for signs of toxicity and mortality. Each animal was given an ophthalmologic examination prior to the first exposure and again during week 12. At the scheduled intervals, after 4 weeks and after 13 weeks of exposure, animals were sacrificed and necropsied. Blood samples were drawn from all animals scheduled for histopathologic evaluation. These samples were used to evaluate eight hematologic and 15 clinical chemistry parameters. Thyroid functions (T3, T4, and TSH serum levels) were also evaluated.

   At necropsy, all animals were examined macroscopically. After 4 weeks of exposure, the following target organs from all animals sacrificed were removed, fixed in formalin, and histologically examined: lungs; lymph node (peribronchial); nasal turbinates; trachea; and thyroid/parathyroid. Absolute organ weights were recorded and relative organ weights (organ-to-body weight ratios) were determined for lungs and thyroid/parathyroid. After 13 weeks of exposure, the absolute and relative weights of 10 organs from all sacrificed animals were recorded. Tissues from the above-mentioned target organs, as well as liver and kidney tissues and all gross lesions and masses were histologically

examined for rats exposed to 20 or 80 mg/m^3. A complete histopathological examination was performed on rats exposed to 0 or 320 mg/m^3, which included tissues of 40 organs and all gross lesions and masses.

Six male and six female rats from each group had previously been designated for residue analysis, and were not sacrificed at study termination. After 13 weeks of exposure, these rats were placed in metabolism cages for 24 hours, during which time the total urine output was collected and frozen. After the 24-hour period, these animals were sacrificed, and the lungs, trachea, and livers were removed and placed in frozen storage. The blood and thyroids were also removed and frozen; the frozen samples and urine were sent to Enviro-Bio-Tech, Ltd. (Bernville, PA) for determination of residual levels of ETU and EBDC as carbon disulfide.

6. The data for body weights, body weight changes, hematologic parameters, clinical chemistry parameters, thyroid function parameters, organ weights, and organ-to-body weight ratios were evaluated using appropriate statistical methods. A difference between exposure groups and controls was considered statistically significant at $p \leq 0.05$.

B. Protocol: The study protocol is included in Appendix A.

12. REPORTED RESULTS:

A. Chamber Conditions: It was reported that the air flow rate during exposure in all chambers was 400 L/min., which resulted in a 99 percent aerosol equilibrium time (t99) of 14.4 min. or 4.0 percent of the exposure time. The chamber concentration and aerosol characterization data are shown in Table 1.

1. 4 Weeks of Exposure: The mean analytical aerosol concentrations of mancozeb in the chambers were found to be 0 (group 1), 22 (group 2), 86 (group 3), and 308 (group 4) mg/m^3; these values corresponded to target concentrations of 0, 20, 80, and 320 mg/m^3. The corresponding respirable concentrations were 0, 8, 40, and 127 mg/m^3 for groups 1, 2, 3, and 4, respectively. The mean mass median diameter (MMD) of the aerosol particles ranged from 3.7 to 4.4 micrometers, the mean geometric standard deviation (GSD) ranged from 2.1 to 2.3, and the respirable fraction ranged from 42 to 47 percent. The mean temperature in the chambers ranged from 20.0 to 21.8°C, and the mean relative humidities ranged from 71 to 74 percent.

2. 13 Weeks of Exposure: The mean analytical aerosol concentrations of mancozeb were 0, 18, 79, and 326 mg/m^3; these values corresponded to target concentrations of 0, 20, 80, and 320 mg/m^3 respectively. The corresponding respirable

TABLE I. Chamber Concentration and Aerosol Characterization

Target Exposure Concentration (mg/m^3)	Mean Analytical Exposure Concentration (mg/m^3)	Range of Daily Exposure Concentration (mg/m^3)	Respirable Exposure Concentration (mg/m^3)	Mean Mass Median Diameter (micrometers)	Geometric Standard Deviation	Respirable Fraction (percent)
After 4 Weeks						
0	0	0	0	0	0	0
20	22	1- 70	8	4.1	2.1	43
80	86	36-152	40	3.7	2.2	47
320	308	161-572	127	4.4	2.3	42
After 13 Weeks						
0	0	0	0	0	0	0
20	18	7- 30	8	3.9	2.1	45
80	79	17-117	36	3.8	2.1	46
320	326	215-514	144	4.2	2.1	42

concentrations were 0, 8, 36, and 144 mg/m^3, respectively. The aerosol particles had mean MMD of 3.8 to 4.2 micrometers, mean GSD of 2.1, and respirable fraction ranged from 42 to 46 percent. The mean chamber temperatures ranged from 19.6 to 21.1°C, and mean relative humidities ranged from 68 to 77 percent.

B. Clinical Observations:

1. 4 Weeks of Exposure: Six animals, distributed among the four groups, died during this study period. The authors attributed these deaths to asphyxiation caused by excessive restraint in the nose-only restraining tubes. Alopecia and missing tail tips were noted for several animals; these findings were attributed to injury by the restraining tubes.

2. 13 Weeks of Exposure: During week 5 to week 13, five additional deaths occurred, two of these were attributed to excessive restraint. One female, exposed to 80 mg/m^3, died as the result of gastric torsion. One male exposed to 320 mg/m^3 died with a prostate abscess. One male exposed to 20 mg/m^3 exhibited gasping, rales, bradypnea, dyspnea, a red serosanguinous exudate on the muzzle, and bright red spotting on the dropping sheet during week 5. The animal appeared emaciated through week 11 with misaligned incisors at week 7, and died during exposure in week 12 with a nasal abscess around a tooth. Several animals exhibited alopecia, dark brown staining of the fur, abrasions, missing tail tips, wet abdominal fur, and/or red exudate around the eyes, all of which were attributed to the restraining method.

C. Body Weights and Body Weight Gains: A summary of the mean body weight data is presented in Table 2.

1. 4 Weeks of Exposure: Mean body weights and mean body weight gains of males exposed to 320 mg/m^3 were significantly reduced ($p \leq 0.05$) compared to controls during weeks 2 through 4. No other exposure-related body weight effects were noted during the first 4 weeks of exposure.

2. 13 Weeks of Exposure: Males exposed to 320 mg/m^3 exhibited significantly reduced ($p \leq 0.05$) mean body weights and mean body weight gains compared to controls during weeks 7 through 13. Females exposed to 20 mg/m^3 exhibited a significantly increased ($p \leq 0.05$) body weight gain as compared to controls. No other exposure-related body weight effects were noted during this time period.

D. Hematology:

1. 4 Weeks of Exposure: An insufficient amount of blood was obtained from 11 of 20 females at the 4-week necropsy interval. The data, therefore, were insufficient to make a meaningful statistical evaluation of female hematologic

TABLE 2. Selected Mean Body Weights (± SD) and Total Body Weight Gain (± SD) in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Group Mean Body Weight (g) at Week				Total Body Weight Gain (Week 0-13)[a]
	0	4	8	13	
Males					
0	182.97 ±12.67	336.4 ±30.0	399.3 ±30.2	360.3 ±40.6	277.1 ±34.0
20	180.27 ±9.83	327.2 ±30.0	383.2 ±40.7	440.1 ±50.6	259.6 ±45.8
80	182.45 ±11.79	329.7 ±22.9	393.3 ±32.1	451.9 ±40.7	271.0 ±37.1
320	181.37 ±10.64	318.0* ±23.3	376.8* ±29.5	429.0* ±34.8	248.3* ±30.5
Females					
0	145.05 ±9.19	200.1 ±17.5	223.0 ±16.3	241.1 ±18.3	95.7 ±16.8
20	142.49 ±9.56	203.3 ±18.3	228.5 ±19.4	251.9 ±25.8	110.1* ±20.1
80	143.92 ±6.19	197.1 ±12.9	225.2 ±14.0	241.6 ±17.4	98.5 ±14.8
320	146.13 ±9.86	205.4 ±14.7	228.4 ±15.7	245.9 ±18.3	99.5 ±14.6

[a]Total weight gain for rats alive at week 13.

*Significantly different from control value ($p \leq 0.05$).

parameters. No hematologic effects were seen for males in any exposure group.

2. 13 Weeks of Exposure: Females exposed to 320 mg/m^3 exhibited a significant increase ($p \leq 0.05$) in mean corpuscular volume (MCV) and a significant decrease ($p \leq 0.05$) in mean corpuscular hemoglobin concentration (MCHC) compared to controls. However, the parameters from which the MCV and MCHC values were derived (hematocrit, red blood cell count, and hemoglobin) were not affected. Therefore, these differences were not considered toxicologically relevant. No other hematologic effects were seen in males or females. Table 3 summarizes mean values of selected hematologic parameters for females.

E. Clinical Chemistry:

1. 4 Weeks of Exposure: No effects were seen in clinical chemistry parameters for males or females in any group after 4 weeks of exposure.

2. 13 Weeks of Exposure: A significant reduction ($p \leq 0.05$) was seen in triglyceride levels in males exposed to 320 mg/m^3 after 13 weeks of exposure. The authors considered this effect to be related to the reduced body weights which were seen these males, and was, therefore, a secondary effect of mancozeb exposure.

   Inorganic phosphorus was significantly reduced ($p \leq 0.05$) in females exposed to 80 mg/m^3 compared to controls. This effect was not observed in females exposed to 320 mg/m^3, and was, therefore, not considered toxicologically relevant by the authors. No other clinical chemistry effects were seen in males or females.

F. Thyroid Function Data:

1. 4 Weeks of Exposure: T3, T4, and TSH serum levels were not affected in males or females of any group during the first 4 weeks of exposure.

2. 13 Weeks Exposure: A significant reduction ($p \leq 0.05$) in T4 serum level was noted in females exposed to 320 mg/m^3; this was considered to be exposure related. No other effects on thyroid function were noted. Female T4 serum levels are presented in Table 4.

G. Residue Analysis (conducted by subcontractor and included in Appendix of report): Urine, blood, and thyroid samples collected after 13 weeks of exposure were analyzed for ETU and EBDC; the data are presented in Table 5. The subcontractor reported that EDBC residues increased in urine at 20, 80, and 320 mg/m^3 and in blood at 320 mg/m^3. The ETU residues increased in urine and blood at 20, 80, and 320 mg/m^3 and in thyroid at 80 and 320 mg/m^3.

TABLE 3. Selected Mean Hematology Values (± SD) in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Parameter/Group Mean Value After 13 Weeks				
	RBC ($10E6/mm^3$)	HCT (%)	HGB (g/100 mL)	MCV (μm^3)	MCHC (%)
Males					
0	8.63 ±0.45	50.4 ±2.0	14.4 ±0.6	58 ±2.0	28.6 ±0.4
20	8.38 ±0.41	49.3 ±0.9	14.1 ±0.7	59 ±2.0	28.5 ±0.8
80	8.47 ±0.34	49.9 ±1.4	14.1 ±0.4	59 ±2.0	28.4 ±0.4
320	8.49 ±0.40	50.0 ±2.9	14.4 ±0.7	59 ±2.0	28.7 ±0.7
Females					
0	7.95 ±0.27	47.9 ±1.7	13.8 ±0.4	60 ±1.0	28.9 ±0.5
20	8.12 ±0.39	49.6 ±2.6	14.1 ±0.7	61 ±1.0	28.5 ±0.4
80	8.09 ±0.40	49.9 ±1.5	14.1 ±0.6	62 ±2.0	28.3 ±0.3
320	7.81 ±0.52	48.5 ±3.6	13.6 ±0.9	62* ±1.0	28.0* ±0.6

*Significantly different from control value ($p \leq 0.05$).

TABLE 4. Selected Thyroid Function Data for Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Group Mean (± SD) T4 Serum Level (µg/dl)	
	After 4 Weeks	After 13 Weeks
Males		
0	4.61 ±1.22	4.39 ±0.76
20	5.40 ±0.75	4.17 ±0.31
80	4.71 ±0.59	4.50 ±0.67
320	4.13 ±1.11	4.02 ±0.60
Females		
0	3.59 ±0.77	3.1[illegible] ±0.60
20	32.6 ±1.13	3.11 ±0.76
80	3.29 ±0.63	2.77 ±0.75
320	2.71 ±0.54	2.18*±0.74

*Significantly different from control value ($p \leq 0.05$).

TABLE 5. Residue Analyses in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Residue Levels (ppm); range or mean ±SD					
	Blood		Urine		Thyroid	
	ETU	EBDC	ETU	EBDC	ETU	EBDC[a]
Males						
0	<0.07 to 0.14	<0.80[b]	0.10±0.07	<0.04 to 0.2	<5.9 to 13	--
20	<0.12 to 0.16	<0.80	0.32±0.22	0.12±0.07	<5.6 to 8.8	--
80	0.18±0.15	<0.80	8.9 ±5.9	0.66±0.5	5.1±2.0	--
320	0.14±0.04	0.86±0.13	13.0 ±9.6	0.53±0.32	7.7±2.5	--
Females						
0	<0.07 to 0.22	<0.80	0.11±0.07	<0.01 to 0.6	<10 to 14	--
20	<0.10 to 0.14	<0.80 to 1.6	0.17±0.68	0.29±0.13	<5.9 to 13	--
80	0.17±0.10	<0.80	16.0 ±8.3	1.3 ±0.83	11.0±5.1	--
320	0.45±0.22	0.91±0.5	69.0 ±66	3.1 ±2.2	28.0±21	--

[a]Not analyzed due to limited sample size.

[b]Values that include symbol for less than (<) indicate the concentration was below th limits of detection.

H. Absolute and Relative Organ Weights:

1. 4 Weeks of Exposure: The authors reported no effects on thyroid or lung weights of males or females in any group after 4 weeks of exposure.

2. 13 Weeks of Exposure: Absolute kidneys and heart weights were significantly reduced ($p \leq 0.05$) in males exposed to 320 mg/m^3 after 13 weeks of exposure. The authors concluded that these reductions resulted from the reduced terminal body weights, and were secondary effects of exposure to mancozeb. No other absolute or relative organ weight effects were observed in either sex in any other group. Table 6 presents mean absolute and relative lung, thyroid, kidney, and heart weights for males and females after 13 weeks of exposure.

I. Ophthalmology: After 12 weeks of exposure to mancozeb, no exposure-related effects were observed in the eyes of any animal in any group. Bilateral retinal degeneration was observed in all of the animals whose cages were in the topmost position on the rack, and was attributed to an excessive amount of room light reaching these cages. One occurrence of ureitis and scattered occurrences of focal retinopathy were noted but were not considered exposure related by the authors.

J. Histopathology:

1. 4 Weeks of Exposure: Several gross and microscopic changes were seen in the tissues examined from the 4-week necropsy. These were scattered among the dosage and control groups, and the authors did not consider any of these changes to be exposure related.

2. 13 Weeks of Exposure: No exposure-related lesions were observed in males or females exposed to 20 mg/m^3 after 13 weeks of exposure to mancozeb. Males and females exposed to 80 or 320 mg/m^3 exhibited yellow-brown granular pigment in the lumen of the cortical tubules of the kidney. The authors considered this to be the result of the elimination of a pigmented metabolite which was considered to be produced as a consequence of exposure, but not toxicologically significant because there were no histopathologic changes seen in the kidney in animals of either group.

   The occurrence of mild hyperplasia of the follicular epithelium in the thyroid glands of three females exposed to 320 mg/m^3 was considered to be related to exposure. No exposure-related lesions were seen in the thyroid glands of male rats.

TABLE 6. Selected Organ Weight Data for Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m^3)	Group Mean Value (± SD) at 13 Weeks							
	Lung		Kidneys		Heart		Thyroid	
	Absolute (g)	Rel. to BW (x 1000)	Absolute (g)	Rel. to BW (x 1000)	Absolute (g)	Rel. to BW (x 1000)	Absolute (mg)	Rel. to BW (x 1000)
Males								
0	2.55±0.29	6.01±0.61	3.54±0.29	8.35±0.69	.64±0.15	3.87±0.31	28.9±4.1	[illegible]
20	2.48±0.29	5.89±0.56	3.25±0.43	7.73±0.83	1.55±0.17	3.64±0.29	26.5±2.4	[illegible]
80	2.67±0.79	6.25±1.86	3.[illegible]±0.29	8.15±0.52	1.61±0.12	3.78±0.27	26.0±5.[illegible]	[illegible]
320	2.44±0.31	6.08±0.64	3.[illegible]±0.32	7.84±0.71	1.48*±0.13	3.70±0.20	26.9±3.3	[illegible]
Females								
0	1.86±0.16	8.53±0.89	2.10±0.13	9.65±0.84	1.00±0.07	4.59±0.51	20.5±3.5	[illegible]
20	1.99±0.35	8.38±1.85	2.20±0.31	9.17±0.93	1.07±0.[illegible]	4.46±0.28	20.6±4.5	[illegible]
80	1.84±0.17	8.20±0.64	2.15±0.30	9.55±1.20	1.00±0.09	4.45±0.33	22.0±2.2	[illegible]
320	2.09±0.38	9.08±1.07	2.32±0.70	10.84±2.48	1.06±0.14	4.64±0.62	22.1±2.4	[illegible]

*Significantly different from control value ($p \leq 0.05$).

Several histopathologic lesions were noted in the respiratory tract, but these were considered to be spontaneous and not related to exposure to mancozeb. Table 7 presents the incidence of findings in the respiratory tract, thyroid, and kidney.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Four groups of 38 male and 38 female rats were exposed to target dust aerosol concentrations of 0, 20, 80, and 320 mg/m^3 for 13 weeks. The actual respirable concentrations for the 13 weeks were 0, 8, 36, and 144 mg/m^3, respectively.

After 4 weeks of exposure, significant ($p < 0.05$) reductions in body weight and body weight gain were noted for male rats exposed to the target concentration of 320 mg/m^3. No other effects were considered to be related to exposure by the authors.

After 13 weeks of exposure, significant reductions were found in body weight and body weight gain in males as well as reductions in T4 levels in females exposed to the target concentrations of 320 mg/m^3. In addition, microscopic examination of the thyroid glands in these females revealed hyperplasia of the follicular epithelium. No other effects were considered to be related to exposure to mancozeb.

There was a yellow-brown granular pigment in the convoluted tubules of male and female rats exposed to 80 or 320 mg/m^3. The authors did not consider this pigment a manifestation of toxicity but likely to be the accumulation of the metabolite, ethylenebisisothiocyanate sulfate.

Based on these results, the NOAEL for mancozeb in rats was considered to be 36 mg/m^3 and the LOAEL was consider to be 144 mg/m^3 based on respirable concentration data.

B. Signed quality assurance statements were dated February 27, 1986 for toxicity and January 13, 1985 for the residue report (Note: this is probably an error in year).

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Considerable variation in chamber concentrations was noted, especially early in this study. The ranges in daily mean chamber concentrations were narrower after the first 4 weeks. The characterization of the dust aerosol was adequate, and it is appropriate to base the toxicological findings on the respirable concentrations of 0, 8, 36, and 144 mg/m^3 for the target concentrations of 0, 20, 80, and 320 mg/m^3, respectively, as reflected in the following assessment.

TABLE 7. Incidence of Selected Histopathologic Lesions Found in Rats Exposed to Mancozeb for 13 Weeks

Organ/Finding	Target Exposure Concentration (mg/m^3)							
	Males				Females			
	0	20	80	320	0	20	80	320
Kidney								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--yellow-brown pigment, cortical tubules	0	0	5	8	0	0	10	9
Thyroid								
No. examined	(10)	(10)	(10)	(11)	(10)	(10)	(10)	(10)
--hyperplasia, follicular epithelium	0	0	0	0	0	0	0	3
Lung								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--multifocal interstitial inflammation	3	3	1	4	9	1	1	8
--foci of alveolar macrophages	5	0	0	3	2	0	1	4
--focal/multifocal hemorrhage	0	0	0	1	0	0	1	0
--diffuse acute congestion	0	1	0	1	0	1	1	1
Nasal Turbinates								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--congestion	0	0	0	0	0	0		
--multifocal mononuclear cellular infiltration	2	2	3	4	0	1	2	2
--hemorrhage	0	0	0	0	0	0	1	1
Trachea								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--focal tracheitis	1	0	1	3	0	2	2	3

The body weight data revealed an exposure-related, significant reduction in body weight and weight gain for male rats exposed to 144 mg/m^3. A significant increase in body weight gain in females exposed to 8 mg/m^3 was also noted; however, these females were slightly smaller at study initiation, and the effect was not seen at higher exposure levels; this weight gain is therefore not considered related to exposure.

The hematology data reflect the general good health of these rats with low white cell counts and no unusual data among the red cell indices. The significant increase in mean corpuscular volume and decrease in mean corpuscular hemoglobin concentration are within the normal range of values for rats and were not considered to be related to exposure to mancozeb.

Triglyceride levels in males exposed to 144 mg/m^3 and inorganic phosphorus levels in females exposed to 36 mg/m^3 were significantly reduced when compared to controls. These changes were within the normal range of values for rats and were not considered to be related to exposure to mancozeb.

The significant reduction in serum T4 levels in females exposed to 144 mg/m^3 noted at week 13 was considered to be related to exposure to mancozeb.

Residue analyses in blood, urine, and thyroids revealed increasing concentrations of ethylenethiourea and ethylenebisdithiocarbamate with increasing exposure concentration. These data support the hypothesis that mancozeb is metabolized to ethylenethiourea and that the thyroid is a target organ for mancozeb toxicity.

The absolute weights of kidneys and heart were significantly reduced in males exposed to 144 mg/m^3. The authors concluded that these differences were due to reduced body weight in these males. However, we calculated organ-to-brain weight ratios and found that the relative kidneys and heart weights were reduced, although not significantly for males exposed to 144 mg/m^3.

No remarkable findings were noted during the ophthalmological examinations.

Microscopic examination of tissues taken at termination (13 weeks) necropsy revealed the presence of yellow-brown, granular pigment in males and females exposed to 36 mg/m^3. The pigment was not present at the 4-week interim sacrifice and, thus, may represent possible progressive lesions in the kidney. In addition, mild hyperplasia of the follicular epithelium of the thyroid occurred in three females exposed to 144 mg/m^3. These changes may be due at least in part to a compensatory reaction to hypothyroidism. Since hyperplasia was not present after the initial 4 weeks of exposure, it is possible that these changes indicate progressive

lesions in female rats. As expected for dust aerosol exposures, there were minor histologic changes in the nasal turbinates and trachea as well as congestion in the lungs of rats exposed to mancozeb. These changes may indicate progressive lung disease leading to the formation of granulomas in the lung and bronchial lymph nodes. Based on these histologic findings, the thyroid, kidneys, and respiratory tract are target organs in rats exposed to dust aerosols of mancozeb.

B. The study was conducted in an acceptable manner and appropriate quality assurance inspections were reported.

C. The effects on body weight in males and on the thyroid in females are similar to effects seen in other studies of mancozeb. The findings of the yellow-brown granular pigment in the kidneys, accompanied by a decrease in kidney weight, has not been reported previously. This may mean that the metabolic fate from inhaled mancozeb is at least slightly different from the fate of ingested mancozeb. The fact that the pigment is granular may indicate a serious kidney problem in rats exposed beyond 13 weeks. These inclusions were not present at the 4-week interim sacrifice while the incidence was high in animals exposed to 36 or 144 mg/m^3. These inclusions may represent a form of kidney urolithiasis as seen with gout, cystinurea, or hyperoxalurea. Stones can form from oxalatea cysteine, uric acid, calcium carbonate, or calcium phosphate. Uric acid is particularly sensitive to pH with stones forming below pH 5.5. The exact nature of mancozeb and/or its metabolites in kidney function is not known; however, these renal inclusions cannot be ignored. An ongoing recovery study will reveal whether or not the pigment is cleared from the kidneys over time. The changes in the respiratory tract are not unexpected for dust aerosol exposures. These types of lesions can progress to the formation of granulomas in the lung and microgranulomas in the bronchial lymph nodes. The ongoing recovery study will give some indications of whether or not these are progressive lesions of the respiratory tract.

Based on these results, there are definite effects of exposure to a respirable concentration of 144 mg/m^3 mancozeb; for males in this study it was body weight and for females effects were on thyroid function and were verified by histology on the thyroid. The seriousness of the renal inclusions (granular pigment) cannot be assessed. The inclusions were not present at 4 weeks, while the incidence was high in the 36 and 144 mg/m^3 groups at study termination. Thus, we cannot ignore inclusions and based on these data, the LOAEL for rats exposed to mancozeb is 36 mg/m^3 and the NOAEL is 8 mg/m^3.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol.

MANCOZEB
SUBCHRONIC INHALATION TOXICITY STUDY IN RATS
PROTOCOL NO. 85P-136
REPORT NO. 86R-000

THIRTEEN-WEEK INTERIM REPORT : 000218

APPENDIX A

STUDY PROTOCOL 85P-136

Mancozeb TH Review 005425

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005425

APPENDIX A

STUDY PROTOCOL 85P-136

005425

1. CHEMICAL: Mancozeb; ethylenethiourea.

2. TEST MATERIAL: Ethylenethiourea prepared by Rohm and Haas, sample TD 83-223, lot No. 08 8-36, had a purity of 99.8 percent; its physical description was not reported.

3. STUDY/ACTION TYPE: Mutagenicity--In vitro transformation assay in C3H 10T/1/2 mouse fibroblasts with ethylenethiourea.

4. STUDY IDENTIFICATION: McGlynn-Kreft, A. M., and McCarthy, K. L. Ethylenethiourea mammalian cell transformation test. (Unpublished study No. 84R-056 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated November 19, 1984.) Accession No. 259044.

5. REVIEWED BY:

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Date: 06-06-86

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Date: 06-06-86 /JEH

128

005425

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NATIONAL SECURITY INFORMATION [EO 12065]

EPA: 68-02-4225
DYNAMAC No. 009-A2
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--In vitro Transformation Assay in C3H/10T 1/2 Mouse Fibroblasts with Ethylenethiourea

STUDY IDENTIFICATION: McGlynn-Kreft, A. M., and McCarthy, K. L. Ethylenethiourea mammalian cell transformation test. (Unpublished study No. 84R-056 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated November 19, 1984.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 4-3-86

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After a 24-hour exposure, the medium was removed and cultures were refed with fresh growth medium. Cells seeded at the lower density were used to determine the plating efficiency. Surviving colonies on these plates were fixed, stained, and counted after 9-10 days of incubation. The remaining cultures, seeded at 2,000 cells/plate, were periodically refed with growth medium throughout the 6-week incubation period. At the conclusion of incubation, cells in the transformation plates were fixed, stained, and scored for transformed foci.

b. Scoring Transformed Foci: Foci were scored by the criteria of Reznikoff et al.[2] as follows:

Type I--Densely stained areas composed of tightly packed cells.

Type II--More densely stained areas than Type I, with piling up of cells and overlapping nuclei.

Type III--Highly polar, piling up of cells, composed of areas exhibiting crisscrossing at the interface of the focus and monolayer.

5. Evaluation Criteria: The assay was considered positive if a dose-response relationship was apparent or the incidence of plates with Type III foci at one dose was significantly higher than the historical untreated and solvent controls.

6. The incidence of plates with Type III foci was statistically compared to the historical untreated and solvent controls by the Fisher Exact test.

7. Evaluation Criteria for Positive Control: Results for the positive control were not analyzed by statistics; the protocol stated that the positive control group must yield an incidence of at least 15 percent of the plates with Type III foci for the positive control to be considered valid.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

Cytotoxicity Assay: The preliminary cytotoxicity assay was conducted with 14 concentrations of the test material ranging from 0.1 to 1000 µg/mL, two doses of the positive control, DMBA, and the solvent

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[2] C. A. Reznikoff, D. W. Brankow, and C. Heidelberger. Establishment and characterization of a cloned cell line C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res. 33(1973): 3231-38.

7. CONCLUSIONS:

A. Under the conditions of this assay and in the absence of S9 activation, ethylenethiourea at doses of 100, 330, and 1,000 µg/mL did not cause an increase in the number of transformed foci in C3H/10T 1/2 cells. The performance of the assay without an exogenous metabolic activation system is an acceptable practice because this cell line can metabolize certain chemicals to active carcinogens. This was adequately demonstrated by the positive response in this assay with the known procarcinogen, 7,12-dimethylbenzanthracene (DMBA).

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Ethylenethiourea, sample TD 83-223, lot No. DB 8-36, had a purity of 99.8 percent and was dissolved in dimethylsulfoxide (DMSO). The stock solution and required dilutions were prepared on the day of treatment.

2. Cell Line: Clone 8 of C3H/10T 1/2 mouse embryo fibroblast cells were obtained from Dr. Charles Heidelberger, University of Southern California, Los Angeles. Logarithmic phase cultures, grown from frozen stock cultures, were used as the target cell.

3. Cytotoxicity Assay: Prepared cells, seeded at a density of 200 cells per plate, were exposed in triplicate to an unspecified number of test material concentrations spanning at least a 4-log dose range. After a 24-hour exposure the medium was removed and cells were incubated [illegible] medium for 9-10 days. Surviving colonies were fixed, stained, counted, and compared to the number of colonies in the solvent control.

4. Cell Transformation Assay: Based on the results of the cytotoxicity assay, three doses were selected for the cell transformation test.

a. Exposure: Prepared cultures, seeded with either 200 or 2,000 cells/plate, were treated with the three selected doses of the test material, solvent, or positive control.

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[1] Only items appropriate to this DER have been included.

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TABLE 1. Representative Results of the C3H/10T 1/2 Transformation Assay with Ethylenethiourea

Substance	Dose (µg/mL)	% Survival[a]	No. Plates w/Type II Foci/ Total No. Replicates	% Replicates w/Type II Foci	No. Plates w/Type III Foci/ Total No. Replicates	% Replicate w/Type II Foci
Negative Control						
Culture Media	--	95	0/20	0	0/20	0
Solvent Control						
Dimethylsulfoxide	--	100	0/30	0	0/30	0
Positive Control						
7,12-dimethyl-benzanthracene	0.5	78	5/20	25	9/20	45[c]
Test Material						
Ethylenethiourea	1000[b]	94	1/80	1.25	0/80	0

[a] $\frac{\text{No. of colonies with test dose}}{\text{No. of colonies with solvent control}} \times 100$

[b] Highest dose tested; values for lower concentrations (100 and 330 µg/mL) we comparable to the solvent control and, therefore, not selected as representative.

[c] Positive by the authors' criterion; ≥ 15% increase in plates with Type III foci.

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control. Cytotoxicity was not evident at any test dose; percent surivival for 1.0 and 2.5 µg/mL DMBA was 59 and 56 percent, respectively.

Transformation Assay: Based on the preliminary cytotoxicity findings, doses selected for the transformation assay were 100, 330, and 1,000 µg/mL. Since cytotoxicity was not achieved, the number of transformation plates for the high dose was increased to 80. For the remaining test doses, media, and positive control, 20 replicates were plated; 30 replicates were used for the solvent control. Plating efficiency was determined from the counts of triplicate plates for all test doses and controls.

The test material was not cytotoxic. No Type III foci were found following exposure of the cells to the three selected doses of the test material; statistical analysis of these data were, therefore, not performed. Representative data from this assay are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "This study demonstrates that ethylenethiourea produces no adverse effects in the Mammalian Cell Transformation Test under the conditions specified."

B. A quality assurance statement was signed and dated November 14, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was properly conducted and the authors' interpretation of the data was correct. Although the test material was not cytotoxic, the assay was conducted at the maximum recommended dose for noncytotoxic compounds.[3] Since no validated exogenous metabolic activation system currently exists for this test, using the C3H/10T 1/2 transformation assay in the absence of S9 activation is acceptable.[4] The sensitivity of the test system to detect the induction of transformants was adequately demonstrated by the positive control (DMBA, 0.5 µg/mL). Hence, the assay system appeared to have the appropriate enzymes to metabolize DMBA to a form that is active for inducing cell transformation.

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[3] C. Heidelberger, A. E. Freeman, R. J. Pienta, A. Sivak, J. S. Bertram, B. C. Casto, V. C. Dunkel, M. W. Francis, T. Kakunaga, J. B. Little, and L. Schechtman. Cell transformation by chemical agents--a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 114(1983): 283-385.

[4] Ibid.

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APPENDIX A
Materials and Methods

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-7; Appendix B, Protocol, CBI pp. 15-17.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION [EO 12065]

EPA: 68-02-4225
DYNAMAC No. 009-A3
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--*In vitro* Transformation Assay for Promotion in C3H/10T 1/2 Mouse Fibroblasts

STUDY IDENTIFICATION: McLeod, P. L. and Doolittle, D. J. Dithane M-45 mammalian cell transformation test for promotion. (Unpublished study No. 84R-297 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated May 29, 1985.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner
Date: 4-3-86

Mancozeb TH Review 005425

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Pages 435 through 445 are not included.

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____ Identity of product inert ingredients.

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7. CONCLUSIONS:

A. Under the conditions of the assay, Dithane M-45 at 0.1 µg/mL did not cause an increase in the number of transformed foci either in initiated or uninitiated C3H/10T 1/2 cells at a presumed maximum tolerated noncytotoxic dose. However, the assay was conducted with only one test dose, which may not be sufficient to conclude that the test material is not an in vitro tumor promoter.

B. The study is unacceptable because only a single dose was used; a single dose is insufficient to show that the test material did not have promoter activity.

8. RECOMMENDATIONS:

Dr. Craig J. Boreiko,[1] a noted expert on initiation/promotion assays, recommends the use of more than one dose level because promoters frequently induce erratic and nondose-related effects. In lieu of established guidelines for initiation/promotion assays, we feel his suggestions should be considered. We, therefore, recommend that the test material should be tested at more than one dose or the authors should justify the use of a single treatment level.

Items 9 and 10--see footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Dithane M-45 (ethylene-bis-dithiocarbamate), lot No. 0842, sample No. TD83-224, had a purity of 88.0 percent and was dissolved in culture medium (Basal Medium Eagles).

2. Cell System: Clone 8 of C3H/10T 1/2 mouse embryo fibroblast cells were obtained from Dr. Charles Heidelberger, University of Southern California, Los Angeles. Logarithmic phase cultures, grown from frozen stock cultures, were used as the target cells.

3. Cytotoxicity Assays:

a. Range-Finding Cytotoxicity Test: Prepared cells, seeded at a density of 200 cells per plate, were exposed in triplicate to six concentrations of the test material.

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[1] Boreiko, C. J., Chemical Institute of Toxicology, Research Triangle Park, NC, personal communications.

[2] Only items appropriate to this DER have been included.

Cells were exposed either for 24 hours or continuously throughout a 9-day incubation period. After the 24-hour exposure, the medium was removed and cells were incubated with growth medium for 8 days. Surviving colonies from both exposures were fixed, stained, and counted.

b. Cytotoxicity Assay with the Initiating Agent: Cultures, seeded at a density of 2000 cells per plate, were exposed to the initiating agent, 0.5 µg/mL N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), for 4 hours. Five days after initiation, cell viability was determined from three control cultures. The remaining cultures were continuously refed with media containing the six selected test doses or the solvent control; viability was monitored daily for 4 days and on alternate days thereafter until termination of the cytotoxicity test, day 11 postinitial compound treatment. A growth curve was plotted (total number of cells vs. days posttreatment) to determine growth inhibition.

4. Cell Transformation Assay for Promotion: Based on the combined results of the cytotoxicity assays, a single test dose was selected for the cell transformation test with promotion.

a. Exposure: The appropriate number of prepared cultures, seeded with either 200 or 2000 cells/plate, was treated with the following agents: 0.5 µg/mL MNNG, 0.5 percent acetone (MNNG solvent), 0.5 µg/mL 7,12-dimethylbenzanthracene (DMBA), or 1.0 µg/mL 3-methylcholanthrene (MCA). Exposure to the initiating agents, MNNG and acetone, was terminated at 4 hours; the exposure period for DMBA and MCA was 24 hours. Five days after initiation, the cultures were refed with media containing the selected dose of the test material, acetone, or the known promoting agent, 0.25 µg/mL 12-o-tetradecanoylphorbol-13-acetate (TPA). This exposure was continued throughout the 6-week promotion phase. The single test material dose was exposed to 20 replicates of untreated cells, 20 replicates of cells preinitiated with MNNG, or 20 replicates of cells pretreated with acetone. The remaining control or TPA-treated groups were similarly added to 20 replicate untreated or preinitiated cultures. Cultures treated with DMBA or MCA were not exposed to promoting agents because the compounds are complete carcinogens. Plating efficiency was not determined for uninitiated or initiated cultures exposed to the test material but was determined for select controls. Surviving colonies on these plates were fixed, stained, and counted after 9-10 days of incubation. Throughout the approximately 6-week promotion phase, cells were continuously refed with media containing the test dose, solvent, or control promoters. At the conclusion of incubation, cells in the transformation plates were fixed, stained, and scored for transformed foci.

b. Scoring Transformed Foci: Foci were scored by the criteria of Reznikoff et al.[a] as follows:

Type I-- Densely stained areas, composed of tightly packed cells.
Type II-- More densely stained areas than Type I, composed of piling up of cells and overlapping nuclei.
Type III-- Highly polar, piling up of cells, composed of areas exhibiting crisscrossing at the interface of the focus and monolayer.

5. Evaluation Criteria: The assay was considered positive if an increase in Type III foci was observed. If an appreciable increase in Type II foci occurred, the test would be repeated with more replicates and/or more or different test concentrations.

6. Evaluation Criteria for Positive Controls: The positive control, DMBA, must yield an incidence of at least 15 percent of the plates with Type III foci to be considered valid evidence of test system sensitivity. Similarly, the promoter control, TPA, must increase the incidence of plates with Type III foci compared to that of the initiating agent alone to be considered acceptable.

7. The data were not statistically analyzed.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

Cytotoxicity Assays:

a. Range-Finding Cytotoxicity Test: The preliminary cytotoxicity assays were conducted with 0.01, 0.033, 0.066, 0.1, 0.25, and 0.5 µg/mL of the test material. Following the 24-hour exposure, cell survival at 0.5 and 0.25 µg/mL was 67 and 83 percent, respectively; below these doses, survival in test groups was comparable to the untreated control. The continuous 9-day exposure resulted in 42 and 76 percent survival at 5 and 0.25 µg/mL, respectively; the remaining doses were not cytotoxic.

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[a] Reznikoff, C. A., Brankow, D. W., and Heidelberger, C. Establishment and characterization of a cloned cell line C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res. 33(1973): 3231-38.

b. Cytotoxicity Assay with Initiating Agent: Cultures preinitiated with 0.5 µg/mL MNNG were continuously exposed to 0.01, 0.033, 0.066, 0.1, 0.25, and 0.5 µg/mL of the test material. Based on the reported growth curve results, an approximately 30 percent inhibition of cells was plotted at day 4 for the highest dose. By day 7, growth at this level was equivalent to the untreated control. At all other concentrations, with the exception of 0.066 µg/mL, cell growth was comparable to the untreated control. The authors did not consider the growth inhibition plotted for 0.066 µg/mL (50 percent at day 4, 30 percent at day 7) treatment related.

Since the cytotoxicity assay with initiated cells showed no definitive cytotoxic effect, the results of the range-finding cytotoxicity tests were used to determine the appropriate dose for the transformation assay.

c. Transformation Assay with Promotion: The selected dose, 0.1 µg/mL, was continuously applied to untreated and acetone- or MNNG-pretreated cells. No foci were observed on untreated or acetone-initiated test plates. Plates of MNNG-initiated, test material-promoted cells contained 10.5 percent Type II foci and 5.3 percent Type III foci. However, the percent Type III foci on these plates was equivalent to the MNNG-initiated, untreated control plates. Representative results are shown in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "This study demonstrates that Dithane M-45 does not promote morphological transformation in the Mammalian Cell Transformation Test for Promotion under the conditions specified."

B. A quality assurance statement was signed and dated May 7, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was properly conducted and the authors' interpretation of the data was correct. No *in vitro* assays to detect tumor promoters have been validated and probably must await the clearer understanding of tumor promotion mechanisms before their use in screening programs can be fully realized.

The authors stated, in accordance with Frazelle et al.,[4] "a nontoxic concentration of test compound is the maximum tolerated dose for assessing promoting activity in this assay."

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[4] Frazelle, J. H., Abernethy, D. J., and Boreiko, C. J. Determination of cell culture conditions optimal for the study of initiation and promotion in C3H 10T 1/2 cells. Environmental Mutagenesis 4(1982): 331-332.

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TABLE 1. Representative Results of the C3H/10T 1/2 Transformation Assay for Promotion with Dithane M-45

Substance[a]		No. Plates w/ Type II Foci/ Total No. Replicates	% Replicates with Type II Foci	No. Plates w/ Type III Foci/ Total No. Replicates	% Replicates with Type III Foci
Initiator	Promoter				
Negative Control					
Media	--	0/20	0	0/20	0
Solvent Control					
Acetone (0.5%)	--	0/20	0	0/20	0
MNNG (0.5 µg/mL)	--	0/20	0	1/20	5
Positive Control					
DMBA (0.5 µg/mL)	--	8/20	40	14/20	70[b]
MCA (1.0 µg/mL)	--	4/20	20	8/20	40[b]
MNNG (0.5 µg/mL)	TPA (0.25 µg/mL)	8/20	40	10/20	50[c]
Test Substance					
Media	Dithane M-45 (0.1 µg/mL)	0/20	0	0/20	0
Acetone (0.5%)	Dithane M-45 (0.1 µg/mL)	0/20	0	0/20	0
MNNG (0.5 µg/mL)	Dithane M-45 (0.1 µg/mL)	2/19[b]	10.5	1/19[d]	5.3

[a]MNNG = N-methyl-N'-nitro-N-nitrosoguanidine
TPA = 12-o-Tetradecanoyl-phorbol-13-acetate
DMBA = 7,12-Dimethylbenzanthracene
MCA = 3-Methylcholanthrene

[b]Positive by the authors' criterion (≥ 15% increase in plates with Type III foci).

[c]Positive by the authors' criterion (increased incidence of plates with Type III foci as compared to initiating agents alone).

[d]One plate not scored; monolayer was not intact.

We confirmed this statement with Frazelle and Boreiko[5] who indicated that the majority of promoters are noncytotoxic. Boreiko[6] recommended, however, that more than a single dose should be assayed (five doses of an unknown agent are routinely evaluated in his laboratory) since promoters frequently induce erratic and nondose-related effects. Although Dithane M-45 was negative at the selected concentration, it is possible that 0.1 µg/mL was not the effective level and tumor promotion could have been detected if more doses were evaluated.

No established guidelines exist for initiation and promotion assays. Since Dr. Boreiko is a recognized expert in this area, his recommendation should be considered in lieu of published guidelines. It is our assessment, therefore, that the results reported by the authors are insufficient to support the conclusion that Dithane M-45 does not promote neoplastic transformation in this assay.

The ability of the known tumor promoter, TPA (0.25 µg/mL), to induce neoplastic transformation in initiated cells was demonstrated. Similarly, the direct induction of transformed clones by DMBA (0.5 µg/mL) and MCA (1.0 µg/mL) was adequately shown. Although no criteria were presented to evaluate a positive effect with MCA, we assumed that the criterion reported for DMBA (≥15% increase in plates with Type III foci) applied to both polycyclic aromatic hydrocarbons.

Item 15--see footnote 2.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-7; Appendix B, Protocol, CBI pp. 16-28.

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[5] Sanchez, J. H. (nee Frazelle) and Boreiko, C. J., Chemical Institute of Toxicology, Research Triangle Park, NC, personal communications.

[6] Ibid.

APPENDIX A

Materials and Methods

Mancozeb TH Review 00425

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Pages 455 through 475 are not included.

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005425

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EPA: 68-02-4225
DYNAMAC No. 009A-4a
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--Salmonella/Mammalian-Microsome Assay

STUDY IDENTIFICATION: Chism, E. M. and Kunze, E. Dithane M-45 microbial mutagen assay. (Unpublished study No. 84R 0059 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 21, 1984.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 4-3-86

476

1. CHEMICAL: Mancozeb; Dithane M-45, coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45 prepared by Rohm and Haas Co., from TD No. 83-224, lot No. 0842, was described as a gray-tan powder with 88 percent active ingredient.

3. STUDY/ACTION TYPE: Mutagenicity--Salmonella/mammalian-microsome assay.

4. STUDY IDENTIFICATION: Chism, E. M. and Kunze, E. Dithane M-45 microbial mutagen assay. (Unpublished study No. 84R 0059 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 21, 1984.) Accession No. 259044.

5. REVIEWED BY:

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Brenda Worthy, M.T.
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Dynamac Corporation

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Date: 4-3-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
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Signature: I. Cecil Felkner
Date: 4-3-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irv Mauer
Date: 04-04-86

Jane Harris
EPA Section Head

Signature: Jay Ellenberger
Date: 04-04-86 JE

7. CONCLUSIONS:

A. Under the conditions of the study Dithane M-45 did not cause increases in reversion in Salmonella typhimurium strains TA1535, TA1537, TA98, or TA100 using an S9 fraction from livers of Aroclor-1254-induced Fischer 344 rats or without activation. The maximum concentration used (250 µg/plate) inhibited growth of each strain with or without S9 activation. The positive controls, 2-anthramine and 2-acetamidofluorene, demonstrated the sensitivity of the assay to detect a mutagenic response with S9 activation only. However, no direct-acting mutagen positive controls were tested to demonstrate the sensitivity of the nonactivated system to detect a mutagenic response.

B. The study was acceptable with S9 activation but unacceptable in the nonactivated assay.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, Dithane M-45, lot No. 0842, was 88 percent active ingredient. Concentration levels were calculated on a basis of 80 percent active ingredient. The diluent for suspension of the test material was water. Water was also the solvent control. The positive controls were 10 µg/plate 2-anthramine for strains TA1535, TA1537, and TA100 and 50 µg/plate 2-acetamidofluorene for strain TA98.

2. S. typhimurium strains TA1535, TA1537, TA98, and TA100 were obtained from Dr. Bruce Ames, University of California, Berkeley, and characterized for growth and histidine requirement at time of transfer. Strains TA98 and TA100 contained PKM 101 R-factor, confirmed by resistance to ampicillin.

3. The S9 preparation was from the livers of Aroclor-1254-induced Fischer 344 rats.

4. Mutagenicity testing was by the method of Ames et al.[2] The test compound was assayed at levels of 2.5, 7.5, 25, 75,

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[1] Only items appropriate to this DER have been included.

[2] Ames, B. M., J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome test. Mutation Research 31(1975): 347-364.

and 250 µg/plate in triplicate with and without S9 activation. Mean and standard deviations of reversions with the solvent control were calculated from 39 plates/strain.

5. Data were analyzed by the method of Mohn and Ellenberger.[3]

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Cytotoxicity: There was inhibition of growth of all the tester strains with Dithane M-45 at concentration levels of 75 or 250 µg/plate as indicated by a decrease in mean number of revertants compared to controls (Table 1).

B. Mutation Assay: There was no increase in revertants compared to controls at 2.5, 7.5, 25, 75, or 250 µg/plate with any of the tester strains. The positive controls gave the expected response in the S9-activated assay. No positive controls were included with the nonactivated assay (Table 1).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Dithane M-45 did not induce reverse mutations in S. typhimurium strains TA1535, TA1537, TA98, or TA100 with or without activation with Aroclor 1254 induced by S9 from liver of Fischer 344 rats. The maximum active ingredient concentration tested, 250 µg/plate, inhibited growth of each of the tester strains.

B. A quality assurance statement was not presented.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the authors' conclusions are supported by the data presented for the S9-activated assay only. It was not stated whether the test compound precipitated in the plates; since the test material is practically insoluble in aqueous or organic solvents, this might have been expected. Cytotoxicity was not measured directly; however, cytotoxicity was evident from the decrease in revertants at 75 and 250 µg/plate when compared to controls. Since no direct-acting mutagens were assayed, the authors failed to demonstrate the sensitivity of the nonactivated system to detect mutagenicity. Therefore, a conclusion cannot be made on the potential of the nonactivated test material to induce mutagenesis in the S. typhimurium tester strains.

---

[3] Mohn, G. R. and J. Ellenberger, in: Handbook of Mutagenicity Test Procedures (eds., Kilbey B. J., M. Legator, W. Nochols, and C. Ramels). Elsevier, Amsterdam, 1977.

TABLE 1. Mutagenicity Assay with Dithane M-45 Using S9 from Fischer 344 Rat Liver

Dose (µg/plate)	Mean Revertants/Plate of Bacterial Tester Strains							
	TA1535		TA1537		TA 98		TA100	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Dithane M-45								
2.5	26.0	20.0	9.7	12.0	25.7	43.7	112.0	100.3
7.5	29.3	16.0	8.3	8.7	32.0	43.0	113.7	89.3
25.0	26.7	11.7	7.0	8.7	26.7	37.7	100.3	89.7
75.0	0.0	7.0	0.0	0.7	3.0	19.3	4.0	21.0
250.0	0.0	5.0	0.0	0.0	0.0	2.3	0.0	0.0
Solvent control	30.5	17.8	9.6	11.4	29.4	52.1	106.6	115.2
Positive control								
2ANTH,[a] 10	32.7	419.0	10.3	176.7			91.2	1457.7
2AAF,[a] 50					30.7	1734.8		

[a]2ANTH is 2-anthramine and 2AAF is 2-acetamidofluorene.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol.

APPENDIX A

Protocol

APPENDIX A

Protocol

Mancozeb T.R. Review 005425

Page ____ is not included in this copy.

Pages 484 through 489 are not included.

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035425

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION [EO 12065]

EPA: 68-02-4225
DYNAMAC No. 009A-4b
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--Salmonella/Mammalian-Microsome Assay

STUDY IDENTIFICATION: Chism, E. M. and Kunze, E. Dithane M-45 microbial mutagen assay. (Unpublished study No. 84R 0060 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 21, 1984.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-3-86

1. CHEMICAL: Mancozeb; Dithane M-45, coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45, prepared by Rohm and Haas Co. from TD No. 83-224, lot No. 0842, was described as a gray-tan powder with 88 percent active ingredient.

3. STUDY/ACTION TYPE: Mutagenicity--Salmonella/mammalian-microsome assay.

4. STUDY IDENTIFICATION: Chism, E. M. and Kunze, E. Dithane M-45 microbial mutagen assay. (Unpublished study No. 84R 0060 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 21, 1984.) Accession No. 259044.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 4-3-86

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 4-3-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-3-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature:
Date: 04-04-86

Jane Harris, Ph.D.
EPA Section Head

Signature:
Date: 04-04-86 JEH

431

7. CONCLUSIONS:

A. Under the conditions of the study, Dithane M-45 did not cause increases in reversion in Salmonella typhimurium strains TA1535, TA1537, TA98, or TA100 using an S9 fraction from livers of Aroclor-1254-induced B6C3F$_1$ mice or without activation. The maximum concentration used (250 µg/plate) inhibited growth of each strain with or without S9 activation. The positive controls, 2-anthramine and 2-acetamidofluorene, demonstrated the sensitivity of the assay to detect a mutagenic response with S9 activation only. However, no direct-acting mutagen positive controls were tested to demonstrate the sensitivity of the nonactivated system to detect a mutagenic response.

B. The study was acceptable with S9 activation but unacceptable in the nonactivated assay.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, Dithane M-45, lot No. 0842, was 88 percent active ingredient. Concentration levels were calculated on a basis of 80 percent active ingredient. The diluent for suspension of the test material was water. Water was also the solvent control. The positive controls were 10 µg/plate 2-anthramine for strains TA1535, TA1537, and TA100 and 50 µg/plate 2-acetamidofluorene for strain TA98.

2. S. typhimurium strains TA1535, TA1537, TA98, and TA100 were obtained from Dr. Bruce Ames, University of California, Berkeley, and characterized for growth and histidine requirement at time of transfer. Strains TA98 and TA100 contained PKM 101 R-factor, confirmed by resistance to ampicillin.

3. The S9 preparation was from the livers of Aroclor-1254-induced B6C3F$_1$ mice.

---

[1]Only items appropriate to this DER have been included.

4. Mutagenicity testing was by the method of Ames et al.[2] The test material was assayed at levels of 2.5, 7.5, 25, 75, and 250 µg/plate in triplicate with and without S9 activation. Mean and standard deviations of reversions with the solvent control were calculated from 39 plates/strain.

5. Data were analyzed by the method of Mohn and Ellenberger.[3]

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Cytotoxicity: There was inhibition of growth of all the tester strains with Dithane M-45 at concentration levels of 75 or 250 µg/plate as indicated by a decrease in mean number of revertants compared to the respective vehicle controls (Table 1).

B. Mutation Assay: There was no increase in revertants compared to controls at 2.5, 7.5, 25, 75, or 250 µg/plate with any of the tester strains. The positive controls gave the expected response in the S9-activated assay. No positive controls were included with the nonactivated assay (Table 1).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Dithane M-45 did not induce mutagenicity in S. typhimurium strains TA1535, TA1537, TA98, or TA100 with or without activation by Aroclor-1254-induced Fischer 344 rat liver S9. The maximum active ingredient concentration tested, 250 µg/plate, inhibited growth of each of the tester strains.

B. A quality assurance statement was not presented.

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[2] Ames, B. M., J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome test. Mutation Research 31(1975): 347-364.

[3] Mohn, G. R. and J. Ellenberger, in: Handbook of Mutagenicity Test Procedures (eds., Kilbey B. J., M. Legator, W. Nichols, and C. Ramels), Elsevier, Amsterdam, 1977.

TABLE 1. Mutagenicity Assay with Dithane M-45 Using S9 from Fischer 344 Rat Liver

Dose (µg/plate)	Mean Revertants/Plate of Bacterial Tester Strains							
	TA1535		TA1537		TA98		TA100	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Dithane M-45								
2.5	29.3	18.7	9.7	10.3	25.7	29.3	125.3	99.7
7.5	25.7	17.3	8.7	9.3	22.0	26.3	120.3	105.3
25.0	30.7	13.0	6.3	7.0	25.3	17.3	100.7	79.7
75.0	0.0	8.0	1.3	0.0	0.7	14.0	5.3	10.7
250.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control	28.7	20.9	8.2	10.5	24.2	33.8	117.7	104.5
Positive control								
2ANTH,[a] 10	31.8	476.2	11.2	180.8			117.7	1099.3
2AAF,[a] 50					21.3	2008.0		

[a]2ANTH is 2-anthramine and 2AAF is 2-acetamidofluorene.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the authors' conclusions are supported by the data presented for the S9-activated assay only. It was not stated whether the test compound precipitated in the plates; since the test material is practically insoluble in aqueous or organic solvents, this might have been expected. Cytotoxicity was not measured directly; however, cytotoxicity was evident from the decrease in revertants at 75 and 250 µg/plate when compared to controls. Since no direct-acting mutagens were assayed, the authors failed to demonstrate the sensitivity of the nonactivated system to detect mutagenicity. Therefore, a conclusion cannot be made on the mutagenic potential of the nonactivated test material in the S. typhimurium tester strains.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol.

APPENDIX A

Protocol

Mancozeb — Tof Reviews 005425

Page ____ is not included in this copy.

Pages 497 through 502 are not included.

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NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-02-4225
DYNAMAC No. 009-A5
April 8, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--Host-Mediated Assay in Mice

STUDY IDENTIFICATION: McCarroll, N. E., and Farrow, M. G. Host mediated assay in mice with compound Dithane M-45. (Unpublished study No. 84RC-25B prepared by Hazelton Laboratories, Vienna, VA, for Rohm and Haas Company, Spring House, PA; dated September 26, 1984.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Cynamac Corporation

Signature: [signature]

Date: 9-8-86

503

1. CHEMICAL: Mancozeb; Dithane M-45, coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45, a pale yellow powder, stored at room temperature, had a purity of 88 percent active ingredient; prepared by Rohm and Haas Co.

3. STUDY/ACTION TYPE: Mutagenicity--host-mediated assay in mice.

4. STUDY IDENTIFICATION: McCarroll, N. E., and Farrow, M. G. Host mediated assay in mice with compound Dithane M-45. (Unpublished study No. 84RC-25B prepared by Hazelton Laboratories, Vienna, VA, for Rohm and Haas Company, Spring House, PA; dated September 26, 1984.) Accession No. 259044.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 4-8-86

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L McLellan
Date: 4-8-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I Cecil Felkner
Date: 4-9-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature: [signature]
Date: 04-11-86

Jane Harris, Ph.D.
EPA Section Head

Signature: [signature]
Date: 4/15/86

7. CONCLUSIONS:

A. Under the conditions of the assay, a definitive conclusion cannot be made regarding the potential of Dithane M-45 for causing a mutagenic response in the host-mediated assay using mice, the dose range selected, 0.5 to 5 mg/kg, was not sufficient.

B. The study is unacceptable.

8. RECOMMENDATIONS:

A. It is recommended that the authors repeat the assay with a dose range that demonstrates some toxic or cytotoxic responses at the highest dose selected, thus demonstrating the appropriateness of the selected dose range.

B. It is also recommended that an evaluation criterion be included in the report.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Dithane M-45 was described as a pale yellow powder, stored at room temperature, with a purity of 88 percent active ingredient. The test material was dissolved in corn oil, the solvent control.

2. Test Animals: Eight-week-old male B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Portage, MI.

a. Animal Maintenance: The animals were acclimated to laboratory conditions for 3 weeks prior to the start of the study. They were individually housed in plastic shoe boxes in an environmentally controlled room (temperature, 73-85°F; relative humidity, 22-39 percent) on a 12-hour light/dark cycle. Food and water were available ad libitum.

b. Group Assignment: Sixty healthy animals were assigned to five treatment groups (20 mice/solvent control group, 10/dose group, and 10/positive control group) using a random number table. Animals were identified by toe clip/ear notch and with a unique species letter.

Items 9 and 10--see footnote 1.

---

[1] Only items appropriate to this DER have been included.

c. Clinical Observation: Animals were observed once on the day of dosing for changes in appearance, behavior, and toxic or pharmacological signs. Body weights were taken prior to dosing.

3. Test Material Preparation and Administration:

a. The test material was prepared fresh on the day of dosing on a weight per volume basis to achieve 100 percent of the active ingredient per dose.

b. The test material at doses of 0.5, 2.0, and 5 mg/kg and the solvent control were administered via oral gavage. The positive control, 10 percent dimethylnitrosamine (DMN), was administered by intramuscular (im) injection.

4. Bacterial Inoculation: Two milliliters of a suspension of Salmonella typhimurium strain TA1530, containing 4.8×10^8 cells/mL, was administered to all animals intraperitoneally (ip) following test material administration.

5. Host-Mediated Assay:

a. Animal Sacrifice: All animals were sacrificed by cervical dislocation. The positive control group was sacrificed 2 hours after bacterial inoculation; the solvent and the three test material groups were sacrificed 4 hours after inoculation.

b. Bacterial Recovery: Animals were cleansed with ethanol and skinned for laparotomy. One milliliter of saline was injected ip through the abdominal musculature wall; the peritoneal cavity was aseptically opened, and the bacterial exudate was withdrawn.

c. Plating of Bacterial Exudate: The standard pour-plate technique was used. For revertant counts, undiluted peritoneal fluid was added in triplicate to complete top agar, containing histidine and biotin, mixed, and poured over Vogel-Bonner E minimal agar. Similarly, for total cell counts, 10-fold dilutions (10^{-1} to 10^{-8}) of peritoneal fluid were prepared in saline and the three highest dilutions were added to top agar and poured over Tryptone Soy Agar plates in triplicate. All plates were incubated at 37°C. After 48 hours, colonies were counted.

6. Evaluation Criteria: No specific criteria for a positive response were reported.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

Clinical observations were reported as normal for all animals, and no deaths occurred during the study. Pretreatment body weights were similar for all groups.

No increase was observed in the mutation frequency of bacterial colonies assayed in mice that were dosed orally with 0.5, 2.0, and 5 mg/kg of the test material when compared to the solvent control. However, the positive control, DMN administered im, caused a 50-fold increase in mutation frequency over the solvent control.

Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Dithane M-45 did not demonstrate a mutagenic response when tested in the Host Mediated mutation assay using Salmonella typhimurium strain TA1530 as the indicator strain and B6C3F$_1$ mice as the host."

B. A quality assurance statement was signed and dated September 26, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the authors interpreted the data correctly and that the test material by oral gavage did not cause a mutagenic response using S. typhimurium strain TA1530 in mice treated with Dithane M-45. By contrast, the positive control by injection caused a 50-fold increase in mutation frequency over the solvent control, therefore demonstrating that the assay system was capable of detecting a mutagenic response. However, the authors did not report any preliminary range finding or cytotoxicity data to support the selection of the dose range used in this assay, nor whether the compound was absorbed from the gastrointestinal tract and reached the indicator organism at an effective concentration. Since the highest dose tested, 5 mg/kg, did not elicit a toxic effect in the dosed mice or a cytotoxic response in the tester strain we concluded that the dose range selected was not high enough to determine the mutagenic potential of Dithane M-45 in the host-mediated assay.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-8; Appendix B, Protocol CBI (Appendix II) pp. 7-13.

TABLE I. Representative Results of the Host-Mediated Assay With S. typhimurium Strain TA1530 in Mice Treated with Dithane M-45

Substance	Dose	Mean MCFU[a] ($\times 10^1$)	Mean CFU[b] ($\times 10^9$)	MF[c] ($\times 10^{-7}$)	Fold Increase Relative to Solvent Control
Solvent control					
Corn oil		5.8	2.95	0.2	-
Positive control					
10% Dimethylnitrosamine	0.1 mL	100.4	0.72	10	50[d]
Test material					
Dithane M-45	5 mg/kg[e]	4.14	2.45	0.2	1

[a]Mean mutant colony-forming units (MCFU)—calculated by reviewers.

[b]Mean colony-forming units (CFU)—calculated by reviewers.

[c]Mutation frequency = $\frac{\text{Mean MCFU}}{\text{Mean CFU}}$

[d]Positive response as reported by authors.

[e]Highest dose tested; lower doses (0.5 and 2 mg/kg) were comparable to the solvent controls.

APPENDIX A

Materials and Methods

Mancozeb — TH Review 005425

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EPA: 68-02-4225
DYNAMAC No. 009-A6
April 8, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--Host-Mediated Assay in Mice

STUDY IDENTIFICATION: [redacted]roll, N.E. Host mediated assay in mice with compound Dithane M-45. (unpublished study No. 85RC-48 prepared by Hazelton Laboratories, Vienna, VA, for Rohm and Haas Company, Spring House, PA; dated July 1, 1985.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 4-7-86

533

1. CHEMICAL: Mancozeb; Dithane M-45, coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45, a pale yellow powder, stored at room temperature, had a purity of 88 percent active ingredient; prepared by Rohm and Haas Co.

3. STUDY/ACTION TYPE: Mutagenicity--host-mediated assay in mice.

4. STUDY IDENTIFICATION: McCarroll, N. E. Host mediated assay in mice with compound Dithane M-45. (Unpublished study No. 85RC-48 prepared by Hazelton Laboratories, Vienna, VA, for Rohm and Haas Company, Spring House, PA; dated July 1, 1985.) Accession No. 259044.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 4-8-86

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 4-8-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I Cecil Felkner
Date: 4-7-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature:
Date: 09-11-86

Jane Harris, Ph.D.
EPA Section Head

Signature:
Date: 4/15/86

7. CONCLUSIONS:

A. Under the conditions of the assay Dithane M-45 at doses of 500, 2000, and 5000 mg/kg did not induce a mutagenic response in the host-mediated assay in mice. The positive control, dimethylnitrosamine (DMN), however, caused a 16.6-fold increase in mutation frequency, demonstrating that the assay had adequate sensitivity to detect a mutagenic response.

B. The study is acceptable.

Items 8 through 10—see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Dithane M-45 was described as a pale yellow powder, stored at room temperature, with a purity of 88 percent active ingredient. The test material was dissolved in corn oil, the solvent control.

2. Test Animal: Six-week-old male B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Portage, MI.

a. Animal Maintenance: The animals were acclimated to laboratory conditions for 13 days prior to study initiation. They were individually housed in plastic shoe boxes in an environmentally controlled room (temperature, 72-78°F; relative humidity, 18-45%) on a 12-hour light/dark cycle. Food and water were available ad libitum.

b. Group Assignments: Sixty healthy animals were assigned to five treatment groups (20 mice/control, 10/dose group, and 10/positive control) using a random card draw. Animals were identified by toe clip/ear notch and with a unique species letter.

c. Clinical Observation: Animals were observed once on the day of dosing for changes in appearance, behavior, once toxic or pharmacological signs. Body weights were taken prior to dosing.

---

[1] Only items appropriate to this DER have been included.

3. Test Material Preparation and Administration:

   a. The test material was prepared fresh on the day of dosing on a weight per volume basis to achieve 100 percent of the active ingredient per dose.

   b. The test material at doses of 500, 2000, and 5000 mg/kg and the solvent control were administered via oral gavage. The positive control, 10% dimethylnitrosamine (DMN), was administered by intramuscular (im) injection.

4. Bacterial Inoculation: Two milliliters of a suspension of Salmonella typhimurium strain TA1530, containing 7.7×10^8 cells/mL, was administered to all animals intraperitoneally (ip) following test material administration.

5. Host-Mediated Assay:

   a. Animal Sacrifice: All animals were sacrificed by cervical dislocation. The positive control group was sacrificed 2 hours after bacterial inoculation; the solvent and the three test material groups were sacrificed 4 hours after inoculation.

   b. Bacterial Recovery: Animals were cleansed with ethanol and skinned for laparotomy. One milliliter of saline was injected ip through the abdominal musculature wall; the peritoneal cavity was aseptically opened, and the exudate was withdrawn.

   c. Plating of Bacterial Exudate: The standard pour-plate technique was used. For revertant counts, undiluted peritoneal fluid was added in triplicate to complete top agar, containing histidine and biotin, mixed, and poured over Vogel-Bonner E minimal agar. Similarly, for total cell counts 10-fold dilutions (10^{-1} to 10^{-8}) of peritoneal fluid were prepared [illegible] highest dilutions were added to top agar and [illegible] Tryptone Soy Agar plates in triplicate. All plates were incubated at 37°C. After 48 hours, colonies were counted.

6. Evaluation Criteria: No specific criteria for a positive response were reported.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

Clinical observations were reported as normal for all animals, and no deaths occurred during the study. Pretreatment body weights were similar for all groups.

No increase in mutation frequency was observed in the assay when mice were given Salmonella ip and dosed orally with 500, 2000, and 5000 mg/kg of the test material when compared to oral dosing with the solvent control. However, the positive control, DMN administered im, caused a 16.6-fold increase in mutation frequency over the solvent control.

Representative results are presented in Table 1.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded that "Dithane M-45 did not demonstrate a mutagenic response when tested in the Host Mediated mutation assay using Salmonella typhimurium strain TA1530 as the indicator strain and B6C3F1 mice as the host."

B. A quality assurance statement was signed and dated May 29, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. It is our assessment that the author interpreted the data correctly and Dithane M-45 did not induce a mutagenic response in the host-mediated assay at doses ranging from 500 to 5000 mg/kg. Although the highest dose tested did not elicit a toxic effect in the mice or a cytotoxic response in the S. typhimurium strain TA1530, the dose selected, 5000 mg/kg, in mice was assessed as more than adequate to test for a mutagenic response. In conjunction with this assessment, in an acute oral LD_{50} in mice with Dithane M-45 fungicide (Rohm and Haas report No. 83R-213A), it was reported that the oral LD_{50} was >5000 mg/kg.

The author did not report an evaluation criterion for determining a positive response; however, the positive control caused a 16.6-fold increase in mutation frequency over the solvent control, demonstrating that the test system was capable of detecting a mutagenic response.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Method, CBI pp. 4-8; Appendix B, Protocol, CBI (Appendix I) pp. 4-10.

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TABLE 1. Representative Results from the Host-Mediated Assay in Mice with Dithane M-45

Substance	Dose	Average MCFU[a] ($\times 10^1$)	Average CFU[b] ($\times 10^{10}$)	MF[c] ($\times 10^{-8}$)	Fold Increase Relative to Solvent Control
Solvent Control Corn oil		10.3	1.90	0.5	—
Positive Control 10% Dimethylnitrosamine	0.1 mL	63.1	0.76	8.3	16.6[d]
Test Material Dithane M-45	5000 mg/kg[e]	10.4	1.8	0.6	1.2

[a] Average mutant colony-forming units (MCFU)—calculated by reviewers.

[b] Average colony-forming units (CFU)—calculated by reviewers.

[c] Mutation Frequency = $\frac{\text{Average MCFU}}{\text{Average CFU}}$

[d] Positive response as reported by the author.

[e] Highest dose tested; lower doses (500 and 2000 mg/kg) were comparable to the solvent control.

APPENDIX A

Materials and Methods

Mancozeb TR Review 005425

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EPA: 68-02-4225
DYNAMAC No. 009A-7
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--CHO/HGPRT Point Mutation Assay

STUDY IDENTIFICATION: Foxall, S., Byers, M. J., and Scribner, H. E. Dithane M-45 CHO/HGPRT gene mutation assay. (Unpublished study No. 84R-207 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated February 11, 1985.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 4-3-86

513

1. CHEMICAL: Mancozeb; Dithane M-45; coordination product of zinc and manganese ethylene-bis-dithiocarbamates.

2. TEST MATERIAL: Dithane M-45, lot No. 0842, TD 83-224, prepared by Rohm and Haas Co., had a purity of 88% active ingredient (ai).

3. STUDY/ACTION TYPE: Mutagenicity--CHO/HGPRT gene mutation assay.

4. STUDY IDENTIFICATION: Foxall, S., Byers, M. J., and Scribner, H. E. Dithane M-45 CHO/HGPRT gene mutation assay. (Unpublished study No. 84R-207 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated February 11, 1985.) Accession No. 259044.

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7. CONCLUSIONS:

A. Under the conditions of the assay Dithane M-45, at doses from 0.5 to 15 µg/mL without activation or at doses from 0.25 to 45 µg/mL with S9 activation prepared from the livers of either Fischer 344 rats or B6C3F1 mice, did not induce a mutagenic response in the CHO/HGPRT gene mutation assay.

B. The study is acceptable under rat or mouse S9-activated test conditions, but unacceptable under nonactivated conditions because the sensitivity of the assay to detect mutagenic events within the range of test material doses was not demonstrated.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1) Test Material: Dithane M-45, lot No. 0842, TD 83-224, had a purity of 88% ai; no further description was reported. The test material was dissolved in distilled water, the solvent control, and test concentrations were prepared fresh for each experiment.

2) Cell Line: The Chinese hamster ovary (CHO) cells used in the study were the BH_4 subclone of the $CHO-K_1$ cell line. Stock cultures were maintained frozen in liquid nitrogen. All cultures, both frozen and growing, were maintained in the absence of antibiotics. Growing cultures were periodically analyzed for mycoplasma contamination, karyotype stability, 6-thioguanine sensitivity, and aminopterin resistance. One week prior to initiation of an assay, cells were grown in Ham's nutrient medium F-12 with hypoxanthine and supplemented with 10% fetal calf serum (heat inactivated).

3) S9 Fraction: The assay was performed with S9 fractions prepared from the livers of both male Fischer 344 rats and B6C3F1 mice induced with Aroclor 1254. The S9 mixes contained per mL: 0.8 mg NADH, 1.2 mg NADP, 1.5 mg G-6-P, 1.05 mg $MgCl_2$, and 1 mg protein/mL of the appropriate S9 fraction.

4) Preliminary Cytotoxicity Study: Cultures, seeded at 5×10^5 cells/plate, were exposed to an unspecified number of test doses for 5 hours with S9 activation or for 18 to 20 hours without activation. These doses spanned a minimum of a 4-log

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[1] Only items appropriate to this DER have been included.

concentration range; parallel cultures were also treated with solvent or positive control chemicals. All cultures were incubated at 37°C, > 90% relative humidity, in 5% CO_2/air atmosphere. Two days after seeding, cells were subcultured with fresh growth medium (hypoxanthine free), and cytotoxicity was determined by the plating efficiency of the test material relative to the solvent control.

5) CHO Mutation Assay: Based on the cytotoxicity data, at least four doses were selected for the CHO assay performed with or without S9 activation. Doses were selected to span a toxicity range of 10 to 90% cell survival. Doses for repeat trials were to be selected on the basis of results from the initial trial.

   a. Treatment: Cells were prepared and treated with the appropriate level of test material, solvent, or positive control with or without S9 activation, as described in the cytotoxicity assay. To terminate exposure the cultures were washed with a saline solution. For cytotoxicity assessment, 200 cells were plated and the remaining cells, seeded at a density of 1×10^6, were subcultured for the mutation expression period.

   b. Mutation Expression Period: Cells used for mutant expression were subcultured twice during the 8-day expression period to maintain cells in logarithmic growth.

   c. Mutant Selection: Selection of 6-thioguanine-resistant mutants ($6TG^r$) was accomplished by plating 2×10^5 cells (five replicates) from each treatment group into media containing 10 µM 6TG. Cell survival (at selection) for each treatment group was assessed from four plates seeded with 200 cells/plate in medium free of 6TG. Selection and survival plates were incubated for 7 days, fixed, stained, and counted. The mutation frequency (MF) was calculated as the number of $6TG^r$ mutants/10^6 survivors.

6) Evaluation Criteria: The test material was considered positive if there was a significant and reproducible dose-related increase in MF relative to the solvent control. If an increase in MF occurred at one dose level, then the result was to be reproduced in an independent assay.

7) Although the statistical methods of Snee and Irr were cited in the references, the data were not statistically analyzed.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. CHO Mutation Assay--Without S9 Activation:

1) Cytotoxicity Study: Dithane M-45 was assayed at nine doses ranging from 0.05 to 1000 µg/mL without S9 activation. Cytotoxicity results, as assessed by plating efficiency, ranged from 88% survival at 0.1 µg/mL to 19% at 5 µg/mL. No cells survived at doses > 10 µg/mL. Dose levels of 0.05 and 0.5 µg/mL were not scored due to the lack of cytotoxicity.

2) Mutation Assay (#1): Based on the cytotoxicity findings, the test material was assayed with duplicate cultures at 0.5, 2, 3, and 6 µg/mL. The results of duplicate cultures were reported separately and referred to as replica 1 or replica 2. Cell survival at the end of treatment for cultures exposed to 6 µg/mL was 55%; below this level, survival was comparable to or higher than the solvent control.

Although the test material did not induce an increase in mutants/10^6 survivors, the toxicity range specified by the protocol (10 to 90% survival) was not achieved in assay #1; therefore, a repeat assay (#2) was performed.

3) Mutation Assay (#2): At test material doses of 5, 7, and 9 µg/mL, survival ranged from 27% at 5 µg/mL (both replicas) to 45% at 9 µg/mL (average of replicas 1 and 2). Since no colonies were found on the test material or the negative control plates, the assay was again repeated (#3); additionally, the protocol requirements for minimum cell survival were not satisfied by assay #2.

4) Mutation Assay (#3): In assay #3, doses of 4, 5, 6, 10, and 15 µg/mL were tested. Average cell survival for both replicas ranged from 49% (4 µg/mL) to 25% (15 µg/mL). Results from replica 1 indicated that an increase in MF (24.7 mutants/10^6) occurred at 10 µg/mL relative to the concurrent solvent control (2.2 mutants/10^6) but not in comparison to the historical control. However, replica 2 for all test doses and the solvent control was lost due to a combination of technical errors, contamination, and cytotoxicity. Since replica 2 was not available, the assay was again repeated (#4).

5) Mutation Assay (#4): Dithane M-45 was assayed at doses of 8, 10, 12, and 15 µg/mL; these doses resulted in an average cell survival range of 28.5% (8 µg/mL) to 9% (15 µg/mL).

In replicas 1 and 2 the MFs for the solvent control were 1.3 and 6.6 $6TG^r$ mutants/10^6. The MFs for the test material at 8 and 10 µg/mL were comparable to the solvent control for both replicates. In replica 1 the MF at 15 µg/ml was 2.2 mutants/10^6. The result obtained in replica 2 at this dose was considered invalid because survival was <5%. There was an increase in MF at 12 µg/mL in replica 1; however, the response was not considered significant because the increase was not confirmed in replica 2.

The positive control, ethyl methanesulfonate (EMS), induced an average MF of 341 $6TG^r$ mutants/10^6 compared to the average MF (3.95/10^6) of the solvent control.

Representative results from the fourth nonactivated assay are presented in Table 1.

B. CHO Mutation Assay--With S9 Activation (Fischer 344 Rat Liver):

1) Cytotoxicity Study: Dithane M-45 was assayed at five doses ranging from 0.1 to 1000 µg/mL in the presence of rat S9 activation. CHO cell survival ranged from 109% at 0.1 µg/mL to 41% at 20 µg/mL. No cells survived at the highest dose tested, 1000 µg/mL.

2) Mutation Assay (#1): Based on the cytotoxicity study, the test material was assayed at doses of 2, 10, 30, 45, and 60 µg/mL. Percent survival at 2 µg/mL was 51% (replica 1) and 33% (replica 2). No cells survived treatment with 60 µg/mL. However, due to extreme toxicity or contamination, the majority of cultures in replica 2 were lost; therefore, the assay was repeated.

3) Mutation Assay (#2): In assay #2, three doses of Dithane M-45 at 0.25, 0.5, and 1.0 µg/mL were tested. Percent survival at all test doses was >60 percent. Although there were no increases in MF induced by the test material in the independent CHO cultures, the positive control, 7-12-dimethylbenzanthracene (DMBA) (7 µg/mL), was nonmutagenic in replica 2. Accordingly, the assay was repeated.

4) Mutation Assay (#3): In assay #3, CHO cells were dosed with 0.5, 1, and 2 µg/mL of the test material. There were no increases in $6TG^r$ MF at any test material dose; however, the MFs for the positive control were low and did not meet assay acceptance criteria. The assay was therefore repeated.

5) Mutation Assay (#4): A fourth rat S9-activated assay was conducted with the test material at doses of 1, 2, 10, 30, and 45 µg/mL. Average relative cell survival ranged from 96% (1 µg/mL) to 53.5% (45 µg/mL).

TABLE 1. Representative Replicate Results from CHO Assay with Dithane M-45 without Activation (Assay #4)

	Dose	Replica	Toxicity after Dosing (% Survival)	Plating Efficiency at Selection (%)	6TG^r Mutant Frequency per 10^6 Survivors
Solvent Control					
Distilled water	--	1	103	78.8	1.3
		2	97	91.3	6.6
Positive Control					
Ethyl methanesulfonate	100 nL/mL	1	62	85.7	301[a]
		2	66	74.2	380[a]
Test Material					
Dithane M-45	12 µg/mL	1	17	90.6	45.2[b]
		2	9	80.9	8.6
	15 µg/mL[c]	1	15	90.1	2.2
		2	3	73.5	0[d]

[a] Significantly positive as assessed by authors.

[b] The increased MF result in replica 1 was not considered significant because it was not confirmed by replica 2.

[c] Highest dose tested; lower doses (from 0.5 to 10 µg/mL) were comparable to the solvent control.

[d] This result was considered invalid because survival was <5%.

In replicas 1 and 2 $6TG^r$ MFs for the solvent control were 5.24 and 6.67/10^6 survivors, respectively. The MFs for the test material at 1, 2, 30, and 45 µg/mL in both replicas were comparable to solvent control values. Although an increase in MF of 33.86 $6TG^r/10^6$ survivors was observed at 10 µg/mL in replica 2; in replica 1, the MF at this dose was 5.78; therefore, the increase was not considered significant because this response was not confirmed. Representative results from the fourth rat S9-activated assay are presented in Table 2.

6) Additional Studies with Various Rat S9 Concentrations: Because there were no mutagenic responses observed with Dithane M-45 in the presence of S9 at 1 mg protein/mL, 1 µg/mL of the test material was assayed using 0.3 and 2 mg protein/mL S9 in duplicate.

Treatment of CHO cells with Dithane M-45 at 1 µg/mL in the presence of S9 at 0.3 mg protein/mL resulted in an MF of 5.35/10^6 survivors (the duplicate was contaminated), and the MF for the solvent control was 1.16/10^6 survivors; however, the positive control was contaminated.

Dithane M-45 at 1 µg/mL in the presence of 2 mg S9 protein/mL resulted in MFs of 10.95 and 7.01 mutants/10^6 survivors; however, the solvent control was contaminated. The MF for the positive control, DMBA, was 252.38 mutants/10^6 survivors. The authors reported, "No difference in mutant frequency was observed at the two different rat S9 concentrations."

C. Mutation Assay--With S9 Activation (B6C3F1 Mice Liver):

1) Cytotoxicity Assay: A cytotoxicity assay, as specified in the protocol, was not performed.

2) Mutation Assay: Dithane M-45 was assayed at 1, 4, 8, 12, and 16 µg/mL with 1 mg S9 protein/mL. The average relative cell survival for the two replicas ranged from 90.5 percent (1 µg/mL) to 5 percent (16 µg/mL).

The MFs of the solvent controls were 4.03 and 4.28 mutants/10^6 survivors in replicas 1 and 2, respectively. Treatment of CHO cells with 1, 4, 8, 12, and 16 µg/mL of the test material induced MFs of 1.06, 0, 5.33, 0, and 0/10^6 in replica 1 and 1.12, 3.20, 1.26, 4.16, and 0 mutants/10^6 survivors in replica 2, respectively; the positive control, DMBA at 7 µg/mL, induced MFs of 141.89 and 97.10 mutants/10^6 survivors.

TABLE 2. Representative Replicate Results from CHO Assay with Dithane M-45 and Fischer 344 Rat Liver S9 Activation (Assay #4)

	Dose	Replica	Toxicity after Dosing (% Survival)	Plating Efficiency at Selection (%)	6TG^r Mutant Frequency per 10^6 Survivors
Solvent Control					
Distilled water	--	1	100	76.4	5.24
		2	58	105.0	6.67
Positive Control					
DMBA[a]	7 µg/mL	1	127	77.9	252.89[b]
		2	125	66.9	218.24[b]
Test Material					
Dithane M 45	10 µg/mL	1	87	86.5	5.78
		2	106	82.7	33.86[c]
	45 µg/mL[d]	1	53	98.1	0
		2	54	65.4	0

[a] DMBA = 7,12-dimethylbenzanthracene.

[b] Significantly positive as assessed by authors.

[c] The increased MF result in replica 2 was not considered significant because it was not confirmed by replica 1.

[d] Highest dose tested; lower doses (from 0.5 to 12 µg/mL) were comparable to the solvent control.

Representative results are presented in Table 3.

3) Additional Studies with Various Mouse S9 Concentrations: Using Dithane M-45 at doses of 4 and 12 µg/mL in the presence of S9 at 0.3 and 2 mg protein/mL, there were no appreciable increases in MFs when compared to the solvent control.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that under the conditions of the study, Dithane M-45 did not induce mutations at the HGPRT locus in CHO cells when cultures were tested in the absence of metabolic activation or in the presence of either Fischer 344 rat liver S9 or B6C3F1 mouse liver S9.

B. A quality assurance statement was signed and dated February 11, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

There were wide variations in cytotoxicity, plating efficiency, and MFs throughout the many repeated studies reported by the authors. We assess, however, that these erratic results were not necessarily indicative of poor laboratory performance, but were probably due to inherent technical problems with the CHO/HGPRT assay. In contrast to mammalian cell assays in suspension (e.g., mouse lymphoma assay), unique problems arise when mutational assays are conducted using monolayer cultures. Several of the major problems outlined below must be considered in order to carefully interpret CHO/HGPRT assay data:

A. Prior to treatment, cells are seeded at a specific density for 24 hours, the assumption being that all cultures grow logarithmically and grow to an equal cell population in all plates by 24 hours. Variability in log-phase growth or cell culture density can, therefore, affect initial and selection cytotoxicity as well as cloning efficiency.

B. Trypsinization of cells after treatment further compromises reproducibility relative to cell survival and cloning efficiency.

C. Reproducibility can also be affected by a low or erratic background MF. In this series of experiments, background MFs ranged from 0 to 21.4 mutants/10^6, which fell within the range considered acceptable by the U.S. Environmental Protection Agency Gene-Tox Program.[2] Therefore, an increase in a test dose

[2] Hsie, A. W., Casciano, D. A., Couch, D. B., Krahn, D. F., O'Neill, J. P., and Whitfield, B. L. The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals, Mutat. Res. 86:193-214, 1981.

TABLE 3. Representative Replicate Results from CHO Assay with Dithane M-45 and B6C3F1 Mice Liver S9 Activation

	Dose	Replica	Toxicity after Dosing (% Survival)	Plating Efficiency at Selection (%)	6TG^r Mutant Frequency per 10^6 Survivors
Solvent Control					
Distilled water	--	1	95	99.3	4.03
		2	105	93.5	4.28
Positive Control					
DMBA[a]	7 µg/mL	1	74	88.8	141.89[b]
		2	90	79.3	9[illegible].10[b]
Test Material					
Dithane M-45	16 µg/mL[c]	1	6[a]	81.1	0
		2	4[b]	79.8	0

[a] DMBA = 7,12-dimethylbenzanthracene.

[b] Significantly positive as assessed by authors.

[c] Highest dose tested; lower doses (from 1 to 12 µg/mL) were comparable to the solvent control.

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with a low background MF may not be reproduced in a followup study if a higher but acceptable background MF is obtained.

The cumulative effects of the major technical problems outlined above are ultimately reflected in MF variations. For example, if plating efficiency is low and mutant counts are within an expected range, the MF will be high not because an induction in mutation has occurred but because of reduced plating efficiency. Therefore, the number of mutants at a given test dose relative to solvent and historical controls assumes greater importance and is probably the crucial parameter for evaluating results.

In light of the above considerations, it is our assessment that the authors interpreted the data correctly and that Dithane M-45 without activation and with rat or mouse S9 activation did not induce a mutagenic response.

The positive controls, EMS and DMBA, demonstrated the sensitivity of the assay to detect a mutagenic response in the absence or presence of S9 activation. However, the dose of EMS (100 nL/mL) used in all nonactivated studies was well above the highest concentration of the test material; therefore, the ability of the nonactivated test system to detect a mutagenic response in a concentration range comparable to the test material was not shown.

The results reported for Dithane M-45 with various rat S9 concentrations were not considered valid because in one assay the positive control was contaminated and in the other assay the solvent control was contaminated; therefore, the reported results could not be evaluated. However, this deficiency did not invalidate this study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-33.
Appendix B, Protocol, CBI pp. 34-47.

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APPENDIX A

Materials and Methods

Mancozeb TH Review 005425

Page ____ is not included in this copy.

Pages 556 through 582 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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EPA: 68-02-4225
DYNAMAC No. 009-A8
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--In vitro Unscheduled DNA Synthesis in Rat Hepatocytes

STUDY IDENTIFICATION: Byers, M. J. and Scribner, H. E. Dithane M-45, in vitro unscheduled DNA synthesis. (Unpublished study No. 84R-280 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated May 29, 1985.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
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Signature: I Cecil Felkner

Date: 4-3-86

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005425

1. CHEMICAL: Mancozeb; Dithane M-45, a coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45, prepared by Rohm and Haas Co. from lot No. 0842, TD No. 83-224, was 88.0 percent active ingredient and described as a yellow powder practically insoluble in water and most organic solvents.

3. STUDY/ACTION TYPE: Mutagenicity--in vitro unscheduled DNA synthesis in rat hepatocytes.

4. STUDY IDENTIFICATION: Byers, M. J. and Scribner, H. E. Dithane M-45, in vitro unscheduled DNA synthesis. (Unpublished study No. 84R-280 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated May 29, 1985.) Accession No. 259044.

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7. CONCLUSIONS:

Unscheduled DNA synthesis (UDS) cannot be adequately evaluated in this study. Dithane M-45 had a presumptive positive response for UDS based on an increase in net nuclear grain counts at 1.0, 2.5, and 5.0 µg/mL; however, the increase was complicated by a rather high cytoplasmic grain count in the solvent control and a decrease in cytoplasmic grain count at the presumptive positive dose levels. The study should be repeated with a different rat hepatocyte preparation.

The study is inconclusive.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A.)

The test compound was uniformly suspended in Williams' medium E without fetal calf serum at 4000 µg/mL, and 15 twofold dilutions were prepared so that when 1 mL of the preparation was added to a culture well containing 3 mL of medium plus fetal calf serum the final concentrations ranged between 0.025 and 1000 µg/mL.

Hepatocytes were isolated from adult male Fischer 344 rats by the method of Williams.[2] Cells (4×10^5) in 4 mL medium were added to 3.5-cm wells of microtiter plates and allowed to attach to plastic coverslips by incubation for 1.5 hours at 37°C. After attachment, medium was removed and the cells were exposed to test compound in 4 mL of medium containing 10 µCi/mL [^{3}H]-thymidine.

The negative controls contained 1 mL of distilled water instead of test compound, and the untreated controls (six wells) received only [^{3}H]-thymidine. The positive control (solvent not stated), 2-acetaminofluorene (2-AAF), was tested at 0.05 and 0.2 µg/mL.

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[1] Only items appropriate to this DER have been included.

[2] Williams, G. M. Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell culture. Cancer Res. 37(1977): 1845-1851.

Following overnight incubation, the cells were washed twice with saline G. Two coverslips from each treatment level were stained with trypan blue for toxicity assessment and four coverslips prepared for radioautography.[a] Slides were stored at -20°C in the dark for 7 days before developing and grain counting.

The assay was considered acceptable if the following criteria were met:

1. The values for the untreated and solvent control were consistent with reported literature values;

2. The positive control values indicated that the hepatocytes were capable of metabolic activation;

3. Slides from at least two treatment concentrations were scorable, and relative survival was greater than 50 percent.

The test material was evaluated positive for UDS if it exhibited a reproducible significant increase in net nuclear grains in the absence of cytotoxicity, as indicated by a decrease in cytoplasmic grains. Two or more consecutive test concentrations must exhibit a significant increase in net nuclear grains. If a positive result occurs at only one concentration, the assay should be repeated with a different hepatocyte preparation at several concentrations around the dose range of the unconfirmed positive to determine if the results were reproducible.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

Cytotoxicity: When Dithane M-45 was tested at 15 dose levels ranging from 0.025 to 1000 µg/mL, the highest dose that had sufficient cells for scoring was 5.0 µg/mL. Treatment concentrations greater than 10.0 µg/mL resulted in cells being stripped from the coverslips. The report stated that viabilities for the treatment groups 24 hours after initiation of treatment ranged from 88.2 percent for the 10-µg/mL group to 99 percent for the 0.025-µg/mL group. Survival relative to the control group is summarized in Table 1. Survival was notably decreased at 10.0 µg/mL.

UDS: Treatment at levels between 1.0 and 10.0 µg/mL produced an increase in net nuclear grains compared to negative controls. However, there was a decrease in cytoplasmic grain counts and an increase in nuclear grain counts.

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[a] Ibid.

TABLE 1. Summary Data for Rat Hepatocyte UDS Assay with Dithane M-45

Treatment Group (µg/mL)	Relative Survival[a]	Average Net Nuclear Grains	Estimated Grain Count[b]	
			Cytoplasm	Nucleus
Solvent Control	115	-4.8	19.8	14.0
	84.4	-5.4	—	—
Untreated Control	71.1	-4.2	—	—
Dithane M-45				
0.25	66.9	-2.6	16.8	14.3
0.50	75.8	-2.1	18.2	16.2
1.00	48.9	3.2	12.2	15.4
2.50	72.0	5.8	12.8	18.8
5.00	75.6	5.5	14.0	19.5
10.00	38.5	4.2	6.4	10.6
Positive Control (2-AAF)				
0.05	62.7	63.3	—	—
0.20	77.7	65.4	—	—

[a]The fraction of attached cells relative to combined solvent controls 24 hours after treatment.

[b]Estimated visually from Figure 1 of report; numerical data were not provided.

Statistical analysis of the data was presented in a memorandum. The data within a slide were not normally distributed, thus it was necessary to log transform the data for statistical analysis. When data were expressed as log (nuclear count/cytoplasmic count), there was a significant increase in the 1.0-, 2.5-, and 5-µg/mL groups ($p \leq 0.01$) compared to controls by groupwise comparison. There was no significant increase in total cell nuclear grain count when the data were log transformed ($p > 0.25$); however, log average cytoplasmic count was significantly decreased compared to controls ($p < 0.05$) at the 1.00- and 2.50-µg/mL levels, but not at the 5.00-µg/mL level. Data for the 10.00-µg/mL level were not included in the statistical analysis because of toxicity and solubility problems.

It was concluded that increases in the net nuclear grain count observed at dose levels of 1.00, 2.50, 5.00, and 10.00 µg/mL resulted from decreases in the cytoplasmic grain counts that were due to cytotoxicity and not to the induction of DNA repair mechanisms.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. When freshly isolated hepatocytes from Fischer 344 rats were incubated for 18 hours in the presence of 10 µCi/mL [^{3}H]-thymidine and various concentrations of Dithane M-45 from 0.025 to 1000 µg/mL, excessive toxicity was found at concentrations above 10 µg/mL and 39 percent survival at 5 µg/mL. No increase in nuclear grains was seen at levels of 0.5, 1.0, 2.5, or 5 µg/mL but there was a dose-related decrease in cytoplasmic grains. Subsequent calculation of net nuclear grains per treatment group resulted in a dose-related increase; however, this did not result from genotoxicity but from cytotoxicity. Dithane M-45 did not induce UDS in isolated Fischer rat hepatocytes.

B. A quality assurance statement was signed and dated May 17, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the results of the assay are inconclusive but indicate a presumptive positive response; the assay should be repeated with a different hepatocyte preparation at levels between 1 and 5 µg/mL Dithane M-45.

The report authors indicated a significant ($p \leq 0.05$) increase in net nuclear grain counts at 1.0, 2.5, and 5.0 µg/mL Dithane M-45 but attributed this increase to a dose-related decrease in cytoplasmic grain counts, which they interpreted to result from cytotoxicity. However, cytoplasmic background counts are in general lower with positive compounds, attributed to active uptake of thymidine into the nuclei of hepatocytes undergoing UDS.

Values for average net nuclear grains for solvent and untreated controls for this study ranged from -4.2 to -4.8. These negative values may be caused by unusually high cytoplasmic grain counts; however, the normal range for values in this testing laboratory was not discussed. Several testing laboratories use a net value of 0 to calculate a mean net nuclear grain count if the cytoplasmic count is greater than the nuclear grain count. Although numerical values for net nuclear grain counts were presented, no data for total nuclear grain counts or cytoplasmic grain count were presented. For this study, the cytoplasmic grain count estimated from Figure 1 of the report was 19.8. The expected value is generally less than 10.[4] When Probst et al. tested UDS with 10 different preparations of rat hepatocytes, in only one preparation was the cytoplasmic mean grain count above 10, and this high value (22) was attributed to insufficient washing of the cells.[5]

Because of these uncertainties, the assay should be repeated with a different preparation of hepatocytes, extra care should be taken in washing the cells, and a solvent other than water should be tested because of the insolubility of the test compound.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-7; Appendix B, Protocol, CBI pp. 17-22.

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[4] Probst, G. S., McMahon, R. E., Hill, L. E., Thompson, C. Z., Epp, J. K., and Neal, S. B. Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. Environ. Mutag. 3(1981): 11-32.

[5] Ibid.

APPENDIX A

Materials and Methods

Mancozeb TR Review 005425

Page ____ is not included in this copy.

Pages 591 through 605 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

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005425

EPA: 68-02-4225
DYNAMAC No. 009-A9
April 4, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--In Vivo Cytogenetic Study in Rats

STUDY IDENTIFICATION: Sames, J. L., McLeod, P. L., and Doolittle, D. J. Dithane M-45 in vivo cytogenetic study in rats. (Unpublished study No. 84R-246 prepared and submitted by Rohm and Haas, Spring House, PA; dated December 21, 1984.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 4-4-86

606

005425

1. CHEMICAL: Mancozeb; Dithane M-45, coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45, prepared by Rohm and Haas Co., TD 83-224, from lot No. 0842, contained 88.0 percent active ingredient.

3. STUDY/ACTION TYPE: Mutagenicity--in vivo cytogenetic study in rats.

4. STUDY IDENTIFICATION: Sames, J. L., McLeod, P. L., and Doolittle, D. J. Dithane M-45 in vivo cytogenetic study in rats. (Unpublished study No. 84R-246 prepared and submitted by Rohm and Haas, Spring House, PA; dated December 21, 1984.) Accession No. 259044.

5. REVIEWED BY:

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6. APPROVED BY:

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Signature: I. Cecil Felkner
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EPA Reviewer

Signature: Irving Mauer
Date: 04-11-86

Jane Harris, Ph.D.
EPA Section Head

Signature: Jane Harris
Date: 04-24-86

607 ✓

7. CONCLUSIONS:

A. Under the conditions of this assay, the acute (one dose) or subacute (daily x 5 days) oral exposure of male rats to the maximum tolerated dose of Dithane M-45 (4400 mg/kg) did not cause a significant increase in chromosomal aberrations in bone marrow cells sampled over the entire mitotic cycle. However, the study was not performed with female animals; therefore, the overall biological significance of these findings cannot be established.

B. The study is adequate for males; but the study as a whole is incomplete because females were not tested.

8. RECOMMENDATIONS: To make this study acceptable, the assay should be conducted using both sexes or the authors should justify the use of a single sex.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Dithane M-45 (ethylene-bis-dithiocarbamate), TD83-224, lot No. 0842, was listed as 88.0 percent pure. The test material was dispersed in corn oil; prepared solutions of the test material were based on the active ingredient content of the test material.

2. Test Animal: Two hundred and forty-eight male Fischer-344 rats, weighing 137 to 158 g, were obtained from Charles River Kingston Breeding Farms, Kingston, NY.

3. Animal Maintenance: Prior to initiation of the study the animals were acclimated to laboratory conditions for 13 days, which included a 6-day quarantine period. Two hundred and forty animals were initially selected and randomly distributed among cages. Throughout the course of the study, animals were housed in an environment controlled for temperature (22.0-25.0°C), relative humidity (43-59 percent), and light (12 hours). With the exception of dosing on day 1, animals were permitted Purina Rodent Lab Chow Checkers ad libitum; water was available ad libitum at all times.

4. Assignment to Groups: One hundred and seventy males, weighing 160.0 to 200.7 g, were selected from the 240 animals. Animals were randomly assigned to treatment groups, housed in groups of two, ear tagged, and identified with a unique number.

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[1]Only items appropriate to this DER have been included.

5. Compound Preparation/Dosing Procedures:

a. Compound Preparation: Based on the active ingredient content of the test material, three concentrations were prepared as corn oil dispersions. The positive control, 1.0 mg/kg triethylenemelamine (TEM), was prepared in distilled water and administered intraperitoneally (ip). Dosing solutions were prepared daily; samples of dosing solutions used on the first and final dosing days were analyzed for test material concentration.

b. Dosing Procedures: The doses of the test material used in this assay (440, 1760, and 4400 mg/kg) were selected based on the highest concentration (5000 and 4400 mg/kg) tested in Fischer-344 rats in an acute oral toxicity study. Toxicity data to support this value were furnished by the sponsor. The appropriate dose levels of the test material, at a dosing volume of 10 mL/kg, were administered orally.

6. Compound Administration:

a. Acute Cytogenetic Study: Thirty animals per group received a single oral administration of the appropriate concentration of the test material or vehicle control. Animals were weighed before dosing and on days 2 and 3; toxic signs were monitored daily. Ten representative members of each group were sacrificed at 6, 24, and 48 hours after compound administration.

The positive control, TEM, was administered as a single dose (1.0 mg/kg, ip) to 10 animals. Animals in this group were sacrificed 18 hours postexposure.

b. Subacute Cytogenetic Study: Ten animals per group were orally administered a single daily dose of the appropriate concentration of the test material or vehicle control for 5 consecutive days. Animals were weighed prior to dosing, observed for toxic effects, and sacrificed 6 hours after the final dose administration.

c. Animal Sacrifice/Bone Marrow Harvest: Colchicine (1 mg/kg, ip) was injected 3 hours prior to the appropriate sacrifice interval; animals were sacrificed by CO_2 asphyxiation. Bone marrow cells were collected from both femurs by aspiration into 0.65 percent KCl. Aspirates were incubated 8-10 minutes at 37°C and centrifuged; the supernatants were discarded. The pellets were fixed three times in methanol:acetic acid (3:1), pipetted onto slides, flame dried, stained, mounted, and coded.

d. Slide Analysis: A maximum of 50 well-defined metaphases per animal were scored for the presence of cytogenetic abnormalities. Chromosomal aberrations were characterized as breaks, gaps, fragments, pulverized cells, translocations, or rearrangements. The number of chromosomes present in each metaphase spread was counted. Gaps were not included in the final analyses.

7. Evaluation Criteria: The data were evaluated for statistical significance ($p < 0.05$) by the Beta Binomial Model[2] and the Fisher Exact Test.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Acute Cytogenetics Study: Toxic signs observed in the acute cytogenetics study following exposure to 4400 mg/kg Dithane M-45 included lethargy observed on day 1 and lethargy, ataxia, dyspnea, stained muzzle, and piloerection observed on day 2. One animal died immediately prior to the day 2 scheduled sacrifice; bone marrow was harvested. With the exception of one animal exhibiting piloerection, all toxicological signs subsided by day 3. Lethargy was only observed 1 day postexposure to 1760 mg/kg Dithane M-45; one animal with a stained muzzle was reported for days 2 and 3. Two animals in the low-dose group had yellow-stained anogenital areas on day 1 and piloerection was recorded for one animal on day 3.

Slight increases in the number of cells with aberrations were noted at the 4400-mg/kg dose after the 24- and 48-hour harvests; however, these increases were not statistically significant. Since no effect was detected following the acute exposure of male rats to 4400 mg/kg Dithane M-45, slides were not scored for the lower doses. Representative results are shown in Table 1. A reduction in analyzable metaphases was observed in the positive control group; however, the percent aberrant cells scored for this group was significantly higher ($p < 0.05$) than the appropriate control exposure group. Based on the results of a dose response study conducted at a later date with 0.1, 0.5, and 1.0 mg/kg TEM ip, the authors concluded that the reduced recovery of acceptable metaphase spreads was due to the higher than expected sensitivity of Fischer-344 rat bone marrow to TEM. Data were furnished to support this conclusion.

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[2] Williams, D. A., "The analysis of binary responses from toxicological experiments involving reproduction and teratogenicity," Biometrics 31 (1975): 949-954.

TABLE 1. Representative Results of the Acute and Subacute In Vivo Cytogenetic Study in Rats After Dithane M-45 Administration

Substance	Dose	Exposure[a] Time	No. of Male Animals Scored	No. of Metaphases Examined	No. of Cells with Aberrations[b]	Percent Aberrant Cells[c]
Vehicle Control						
Corn oil	10 mL/kg	6 h	10	500	2	0.4
		24 h	10	465	1	0.2
		48 h	10	500	6	1.2
		5 d	10	500	3	0.6
Positive Control						
Triethylenemelamine	1.0 mg/kg	18 h	10	85[d]	54	63.5[e]
Test Material						
Dithane M-45	4400 mg/kg[e]	6 h	10	477	2	0.4
		24 h	10	477	4	0.8
		48 h	10	500	8	1.6
		5 d	7	350	2	0.6

[a] Time after compound administration.

[b] Gaps not included.

[c] Percent aberrant cells = $\frac{\text{No. of cells with aberrations}}{\text{No. of metaphases examined}} \times 100$

[d] Reduced recovery of acceptable metaphase spreads.

[e] Toxic signs observed at all observation intervals; three animals died in the subacute study.

[f] Significantly different from the 24-hour control value at $p < 0.05$ by Fischer Exact Test and Beta Binomial Model

Note: Chromosome preparations for the 1760- and 440-mg/kg dosing groups were not scored.

B. Subacute Cytogenetic Study: Five consecutive daily administrations of 4400 mg/kg Dithane M-45 resulted in overt toxicity that intensified as the sequential dosing regime progressed. By day 4, two animals exposed to 4400 mg/kg Dithane M-45 were dead, 90 percent were lethargic, 80 percent exhibited piloerection, and 30 percent were dyspneic. Other toxic signs included stained muzzles, ataxia, diarrhea, and yellow-stained anogenital areas. Toxic signs reported for day 5 occurred at a comparable or in some cases lower frequency than day 4; one animal was found dead. The cumulative toxic effects of the mid dose (1760 mg/kg) were not readily apparent until day 3 (70 percent with piloerection). By day 5, all animals were lethargic, showed piloerection, and 40 percent had stained muzzles. The continuous 5-day exposure to 440 mg/kg Dithane M-45 resulted in animals with lethargy (days 1 and 5) and piloerection (100 percent on days 4 and 5).

No statistically significant increase in chromosomal aberrations resulted from the subacute exposure of the male rats to 4400 mg/kg Dithane M-45. Slides were not scored for the mid and low doses. Representative results are present in Table 1.

C. Dosing Solution Analysis:

Results of analyzing the dosing solution indicate that the dosing solutions prepared on days 1 and 5 were within 1 percent of the expected concentration.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "No adverse effect occurred in bone marrow chromosomes of rats following either an acute or subacute dosing regime of Dithane M-45. Therefore Dithane M-45 does not represent a cytogenetic hazard under the conditions of this test."

B. A quality assurance statement was signed and dated December 30, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that this study was well conducted and the authors' interpretation of the data was correct. Dose-related toxicological signs were evident in animals exposed to the three doses of the test material in both the acute and subacute dosing regimes, indicating that the selected dose range was appropriate for this study. However, no explanation for using a single sex was provided. Unless preexisting evidence suggested that adverse compound effects were sex related, it would have been prudent to perform this study with an equal sex distribution.

Although a reduced recovery of analyzable metaphase spreads for the positive control group was reported by the authors, the statistically significant increase in chromosomal aberrations in rats treated with the positive control (TEM, 1.0 mg/kg, ip) adequately demonstrated the sensitivity of the test system to detect cTastogenic agents. The authors further demonstrated in a later study that the reduction was related to the sensitivity of Fischer-344 rats to TEM.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 1-7; Appendix B, Protocol, CBI pp. 15-27.

APPENDIX A

Materials and Methods

Mancozeb Mf Review 005425

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Pages 615 through 635 are not included.

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____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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EPA: 68-02-4225
DYNAMAC No. 009-A10
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells

STUDY IDENTIFICATION: Ivett, J. L. and Myhr, B. C. Mutagenicity evaluation of Dithane M-45 fungicide lot No. 0842 in an *in vitro* sister chromatid exchange assay in Chinese hamster ovary (CHO) cells. (Unpublished study No. 84RC-60 prepared by Litton Bionetics, Inc., Kensington, MD, for Rohm and Haas, Spring House, PA; dated March 1985.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-3-86

1. CHEMICAL: Mancozeb; Dithane M-45, coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45, a yellow-powdered fungicide prepared by Rohm and Haas Co., was obtained from lot No. 0842 (TD 83-224); its purity was not reported.

3. STUDY/ACTION TYPE: Mutagenicity--sister chromatid exchange assay in Chinese hamster ovary (CHO) cells.

4. STUDY IDENTIFICATION: Ivett, J. L. and Myhr, B. C. Mutagenicity evaluation of Dithane M-45 fungicide lot No. 0842 in an in vitro sister chromatid exchange assay in Chinese hamster ovary (CHO) cells. (Unpublished study No. 84RC-60 prepared by Litton Bionetics, Inc. Kensington, MD, for Rohm and Haas, Spring House, PA; dated March 1985.) Accession No. 259044.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
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Date: 4-3-86

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Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irving Mauer
Date: 04-04-86

Jane Harris, Ph.D.
Acting EPA Section Head

Signature: Jane Harris
Date: 04-04-86

7. CONCLUSIONS:

A. Under the conditions of this assay and in the absence of S9 activation, Bithane M-45 at doses of 7.5, 10.0, 12.5, and 15.0 µg/mL in the initial study and 15.0 and 17.5 µg/mL in a repeat assay induced statistically significant and dose-related increases in the incidence of sister chromatid exchanges (SCEs) per metaphase in Chinese hamster ovary (CHO) cells in vitro. Under conditions using mouse S9 activation, doses ranging from 10 to 17.5 µg/mL induced significant increases, which, however, were not dose related or confirmed in a repeat study. We assess that the test material produced a positive response for induction of SCE in the nonactivated assay and a presumptive, but inconclusive, positive response in the S9-activated system.

B. The study is acceptable.

Items 8 through 10—see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details).

1. Test material: Dithane M-45, a fungicide, was obtained from lot No. 0842 (TD 83-224) and was described as a yellow powder with an unspecified purity. At the request of the sponsor, the test material was suspended in serum-free culture medium, the solvent of choice for this study.

2. Cell line: Chinese hamster ovary (CHO) cells, CHO-WB1, were obtained from Dr. S. Wolff, University of California, San Francisco. CHO cells used in this assay were grown for 24 hours in McCoy's supplemented medium prior to use.

3. The S9 fractions used for metabolic activation were prepared from the livers of Fischer 344 rats and B6C3F1 mice induced with Aroclor 1254.

4. Preliminary Cytotoxicity Assays:

a. Relative Growth Assay: Prepared cells (0.3×10^6 cells/25 cm^2 flask) were exposed to 10 doses of the test material for 2 hours in the absence or presence of rat and mouse S9 fractions. Cells were washed, incubated in fresh medium for 24 hours, and counted with a Coulter counter, and percent growth, relative to the solvent and the two lowest doses of the test material, was calculated.

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[1]Only items appropriate to this DER have been included.

b. Relative Cloning Efficiency: Cells were seeded at a density of 200 cells per plate, allowed a 16- to 18-hour attachment period, and exposed to 10 concentrations of the test material for 2 hours in the absence or presence of rat and mouse S9 fractions. Treated cells were washed, incubated in media for 7 days, and counted, and the number of colonies in dosed groups was compared to the control cultures.

5. Sister Chromatid Exchange (SCE) Assay:

a. Exposure: Exponentially growing cells, seeded at 1×10^6 per flask, were exposed in duplicate to three to five doses of the test material solvent or positive controls for 2 hours in the presence or absence of the appropriate S9 fraction. Cells were rinsed, refed media containing 10 µM BrdU, and incubated 26 hours. Colcemid (0.1 µg/mL) was added during the last 2-2.5 hours of incubation. Cytotoxicity was assessed in duplicate flasks for each test dose concurrent with the SCE assay.

b. Preparation of Chromosomes: Cells were collected by mitotic shake-off, treated with hypotonic solution, fixed, dropped onto slides, air dried, stained by a modified fluorescent-plus-Giemsa technique, mounted, and coded.

c. Slide Analysis: A maximum of 50 well-defined metaphases (25 cells/flask) were scored from the four highest doses of test material and the negative control; 25 cells were scored for the positive control.

6. Evaluation Criteria: The assay was considered positive if a) an approximate doubling in SCE frequency over the negative control was observed at one or more doses or b) in the absence of a doubling, if a statistically significant increase occurred at a minimum of three doses.

7. A t-test was used to statistically analyze the data.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assays:

1. Relative Growth Assay: Doses of the test material ranging from 1 to 100 µg/mL were tested with or without rat and mouse S9 activation. Under all conditions of activation or nonactivation, relative survival increased as the test material concentration was decreased. The test material was

more cytotoxic at higher concentrations (≤10 percent survival at 20-100 µg/mL) without S9 activation. Slightly higher survival (8 to 12%) at comparable doses was recorded in the presence of mouse S9; however, at 10 µg/mL, 39 percent of the cells survived compared to 65 percent in the nonactivated assay. Cytotoxicity was markedly diminished in the presence of rat liver S9; survival ranged from 20 percent at 100 µg/mL to 71 percent at 20 µg/mL.

2. Relative Cloning Efficiency: Similar concentrations (1-100 µg/mL) were assayed with or without mouse and rat S9 to determine relative clonal survival. Cytotoxicity in suspension cultures as assessed by survival was more severe than in plated cells. No colonies were recovered from cells treated with doses up to and including 6.0 µg/mL under activated or nonactivated conditions. Percent survival at 4.0 µg/mL was 8.3 percent (-S9), 0 percent (+ mouse S9), and 7.9 percent (+ rat S9). At the two remaining doses (1 and 2 µg/mL) and in agreement with the relative growth assay findings, cytotoxicity was most pronounced without S9 activation and least apparent in the presence of rat-induced S9 fraction. Representative results from the two cytotoxicity assays are presented in Table 1.

B. SCE Assay: Based on the combined findings of the cytotoxicity assays, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 µg/mL were investigated in the nonactivated and rat or mouse S9-activated SCE assay. A suspension cytotoxicity assay with the above concentrations and under the same conditions was conducted in parallel with the SCE assay; cells were visually examined for mitotic delay.

1. Nonactivation Assay: Following exposure to the selected test doses (2.5 to 20 µg/mL), relative growth ranged from 4 to 24 percent at the five highest doses (20-10 µg/mL). At doses below 10 µg/mL, >50 percent of the cells were viable. Metaphases harvested from CHO cells treated with 5, 7.5, 10, 12.5, and 15.0 µg/mL were examined for mitotic delay and frequency of SCEs. At the three highest test material doses, the average percentage of first division metaphases (92 percent at 15.0 µg/mL, 75 percent at 12.5 µg/mL and 82 percent at 10.0 µg/mL) indicated that progression through the mitotic cell cycle was delayed. Cell cycle kinetics were not severely affected by 7.5 and 5 µg/mL. Due to extreme cytotoxicity at 15.0 µg/mL, only 38 metaphases were scored; however, a statistically significant increase in SCEs/cell (1.9 times higher than control) was reported. Significant and dose-related increases were also observed at doses of 12.5, 10.0, and 7.5 µg/mL. An elevated but not significant increase was reported at the lowest dose, 5.0 µg/mL. Results from analyzed nonactivated doses are shown in Table 2.

TABLE 1. Representative Results of the Relative Growth (Suspension) and Relative Clonal Survival Cytotoxicity Assays with Dithane M-45 (Chinese Hamster Ovary Cells)

Substance	Dose Unit/mL)	Activation Condition: -S9 % RS[a]	-S9 % CS[b]	+ Mouse S9 % RS[a]	+ Mouse S9 % CS[b]	+ Rat S9 % RS[a]	+ Rat S9 % CS[b]
Negative Control							
Culture Media		100	100	100	100	100	100
Test Material							
Dithane M-45	1	100	76.0	100	111.1	100	100.4
	2	100	43.7	100	72.2	100	82.2
	4	104	8.3	—[c]	0	92	7.9
	6	94	0	74	0	85	0
	8	84	0	78	0	90	0
	10	65	0	39	0	67	0
	20	10	0	12	0	71	0
	40	4	0	11	0	77	0
	60	2	0	9	0	40	0
	100	4	0	8	0	20	0

[a] % RS--Relative survival derived from mean value of negative control and 1 and 2 µg/mL of the test material.

[b] % CS--Clonal survival; % CS relative to negative control.

[c] No value, culture contaminated.

TABLE 2. Representative Results from the Nonactivated Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells Treated with Dithane M-45

Substance	Dose (unit mL)	% Relative Survival	No. Metaphases Scored	Average % M_1 Cells[a]	Average % M_2 Cells[a]	No. SCEs/cell ±SD	Fold Increase in SCEs[b]
Negative Control							
Culture Media	—	100	50	10	90	8.7±0.4	—
	—[c]	ND[d]	50	14	86	7.5±0.4	—
Positive Control							
Mitomycin C	10 ng	ND	25	8	92	13.1±0.9*	1.5
	10 ng[c]	ND	25	17	83	10.0±0.6*	1.3
Test Material							
Dithane M-45	5.0 µg	108	50	28.3	71.7	10.5±0.5	1.2
	7.5	58	50	55.0	45.0	13.0±0.7*	1.5
	10.0	24	50	82.0[e]	18.0	14.2±0.8*	1.6
	12.5	16	50	75.0[e]	25.0	14.3±1.0*	1.6
	15.0	11	38	92.0[e]	8.0	16.5±1.0*	1.9
	10.0[c]	ND	50	42.0	58.0	8.4±0.4	1.0
	12.5	ND	50	41.5	58.5	8.7±0.5	1.2
	15.0	ND	50	35.0	65.0	10.0±0.6*	1.3
	17.5	ND	50	43.5	56.5	10.0±0.6*	1.3

[a]Averaged by our reviewers; M_1=first division metaphases and M_2=second division metaphases.

[b]Fold increase in SCEs = $\frac{\text{No. of SCEs/cell (treatment group)}}{\text{No. of SCEs/cell (negative control)}}$; calculated by our reviewers.

[c]Repeat study.

[d]ND—Not determined.

[e]Marked increase in M_1 cells indicative of mitotic delay (cytotoxicity).

*Significant increase ($p < 0.05$) by t-test.

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Based upon the evidence of a significant and dose-related positive effect and at the request of the sponsor, the nonactivated SCE assay was repeated with 5, 10, 12.5, 15, and 17.5 µg/mL. A relative growth assay was not performed; the integrity of the monolayers and the degree of cell replication were determined qualitatively. Although there was no reduction in monolayer confluency, cell cycle delay was observed at test material doses of 15 and 17.5 µg/mL. Accordingly, cells treated with 12.5, 15, and 17.5 µg/mL were permitted a longer expression in the presence of BrdU. Following the additional incubation, metaphases were scored for all doses except the low dose (5 µg/mL). Slight mitotic delay was still apparent at all doses. Significant increases in the frequency of SCEs were reported for the 15.0 and 17.5 µg/mL dose levels. From the initial findings and the confirming results of the second nonactivated SCE assay, the authors concluded that Dithane M-45 was positive in the nonactivated assay. Representative results from all analyzed doses in the repeat study are presented in Table 2.

2. Activated Assays:

   a. Mouse S9 Activation: In the presence of mouse liver microsomes, relative survival ranged from 4 to 92 percent over concentrations spanning 20.0 to 2.5 µg/mL. Due to the extreme cytotoxicity at 20.0 µg/mL, no metaphases were obtained. The four intermediate doses that were scored (10.0, 12.5, 15.0, and 17.5 µg/mL) showed no definitive evidence of cell cycle delay. At these levels, statistically significant increases in SCEs per cell were observed. However, they were not dose related. The assay was repeated with 12.5, 15, 17.5, and 20.0 µg/mL. Confluency of the monolayers and cell replication were determined qualitatively. At 15 and 20 µg/mL, monolayer confluency was reduced and mitotic delay was observed. Expression time for these cultures in the presence of BrdU was extended and metaphases were scored at 12.5 through 20.0 µg/mL. The significant, but not dose-related, increases in SCEs per cell reported in the initial mouse S9-activated test were not reproduced. The authors concluded that the test material under mouse S9-activated conditions was not genotoxic. However, the comparative response of CHO cells to the activated positive control, cyclophosphamide (1.5 µg/mL), indicated that in the repeat study the number of SCEs induced by cyclophosphamide was approximately 50 percent lower than in the initial trial. Representative results from all analyzed doses in the initial and repeat studies are shown in Table 3.

TABLE 3. Representative Results from the Mouse S9-activated Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells Treated with Dithane M-45

Substance	Dose (µg/mL)	% Relative Survival	No. Metaphases Scored	Average % M_1 Cells[a]	Average % M_2 Cells[a]	No. SCEs/cell ±SD	Fold Increase in SCEs[b]
Negative Control							
Culture Media	—	100	50	3.0	97.0	9.0±0.5	—
	—[c]	ND[d]	50	16.5	83.5	8.8±0.4	—
Positive Control							
Cyclophosphamide	1.5	ND	25	ND	ND	60.2±2.0[e]	6.7
	1.5[c]	ND	25	14.0	86.0	25.2±1.0[e]	2.9
Test Material							
Dithane M-45	10.0	32	50	27.5	72.5	12.8±0.6[e]	1.4
	12.5	22	50	40.5	59.5	14.5±0.8[e]	1.6
	15.0	18	50	32.0	68.0	11.0±0.5[e]	1.2
	17.5	16	50	36.0	64.0	13.6±0.6[e]	1.5
	12.5[c]	ND	50	73.5	26.5	9.2±0.4	1.0
	15.0	ND	50	44.5	50.5	9.6±0.5	1.1
	17.5	ND	50	43.5	56.5	8.3±0.4	<1.0
	20.0	ND	50	43.5	56.5	9.3±0.5	1.0

[a]Averaged by our reviewers.

[b] Fold Increase in SCEs = $\frac{\text{No. of SCEs/cell (treatment group)}}{\text{No. of SCEs/cell (negative control)}}$; calculated by our reviewers.

[c]Repeat study.

[d]ND - Not determined.

[e]Significant increase ($p < 0.05$) by t-test.

b. Rat S9 Activation: Results from the rat liver S9-activated assay with eight doses (2.5-20 µg/mL) indicated that Dithane M-45 was less toxic in the presence of rat S9. At 20.0 µg/mL, 36 percent of the CHO cells survived; percent survival for the remaining doses ranged from 34 percent at 17.5 µg/mL to 91 percent at 2.5 µg/mL.

Metaphases from cells exposed to 12.5, 15, 17.5, and 20 µg/mL were analyzed for SCE frequencies. An evaluation of cell cycle kinetics showed that a slight depression of second division metaphase cells occurred at all dose levels; the reduction was not sufficient to extend the expression period. A statistically significant but less than doubling of the SCE frequency was noted at 17.5 µg/mL. Although the remaining doses had elevated counts, no significant increases were observed. Representative results are presented in Table 4.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "Given the repeatable positive response under -S9 conditions, the test article is considered positive for inducing sister chromatid exchange under the conditions of this assay."

B. A quality assurance statement was signed and dated September 4, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was conducted properly and that the authors' interpretation of the data was correct. Statistically significant, dose-related, and reproducible increases in SCE frequency were demonstrated under nonactivated conditions. The lack of reproducible, significant increases in the mouse S9-activated assays may have been related to reduced sensitivity of the CHO cells in the repeat test. As shown in Table 3, the increase in SCEs from the cyclophosphamide treatment in the repeat assay (2.9 times) was approximately half of the response reported in the initial assay (6.7 times). Similarly, the percent second division metaphase cells for the negative control in the second assay was lower (83.5%) than in the first assay (97.0%). These two factors may have contributed to reduced cell sensitivity and, therefore, could have obliterated subtle effects induced by mouse-activated Dithane M-45. However, had the authors been able to reproduce the significant increases observed in the first trial, it is doubtful that the effect could be considered a definitive positive response. The increases for both the initial and repeat

TABLE 4. Representative Results from the Rat S9-activated Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells Treated with Dithane M-45

Substance	Dose (μg/mL)	% Relative Survival	No. Metaphases Scored	Average % M_1 Cells[a]	Average % M_2 Cells[a]	No. SCEs/cell ±SD	Fold Increase in SCEs[b]
Negative Control							
Culture Media	—	100	50	1	99	9.6±0.5	—
Positive Control							
Cyclophosphamide	1.5	ND[c]	25	3	97	29.9±1.4*	3.1
Test Material							
Dithane M-45	12.5	51	50	50.0[d]	51.0[d]	10.2±0.5	1.1
	15.0	42	50	33.5	66.5	10.8±0.5	1.1
	17.5	34	50	45.0	55.0	11.6±0.4*	1.2
	20.0	36	50	42.0	58.0	10.3±0.5	1.1

[a]Averaged by our reviewers.

[b] Fold increase in SCEs = $\frac{\text{No. of SCEs/cell (treatment group)}}{\text{No. of SCEs/cell (negative control)}}$; calculated by our reviewers.

[c]ND—Not determined.

[d]Reported values $M_1 + M_2 > 100\%$.

*Significant increase ($p < 0.05$) by t-test.

mouse-activated assays satisfied neither the reporting laboratory's nor the USEPA Gene-Tox Program's[2] criteria for a positive response in this assay. Criteria established by the USEPA Gene-Tox Program for evaluating a positive effect require:

A. The ability to induce at least a twofold increase over baseline SCE frequencies or

B. The demonstration of a three-point, dose-response curve showing a progressive increase over baseline SCE frequencies with at least one SCE value at $p < 0.001$ level.

It is our assessment, therefore, that the test material is genotoxic in the absence of S9 activation; the inability to reproduce significant responses induced by mouse S9 activation did not alter the overall conclusions presented by the authors.

The significant increases elicited by the positive controls (Mitomycin C, 10 ng/mL -S9; cyclophosphamide, 1.5 µg/mL + mouse or rat S9) adequately demonstrated the sensitivity of the test system to detect genotoxic activity. Although a reduction in assay sensitivity was observed in the repeat mouse S9-activated test, the effect induced by cyclophosphamide was significant.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods (Protocol), CBI pp. 26-32.

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[2] Latt, S. A., Allen, J., Bloom, S. E., Carrano, A., Falke, E., Kram, D., Schneider, E., Schreck, R., Tice, R., Whitfield, B., and Wolff, S. Sister-Chromatid Exchanges: A Report of the Gene-Tox Program. Mutation Research 87(1981): 17-62.

APPENDIX A

Materials and Methods (Protocols)

Mancozeb — TF Review 005425

Page ____ is not included in this copy.

Pages 649 through 655 are not included.

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005425

EPA: 68-02-4225
DYNAMAC No. 009-A11
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--*In vitro* Transformation Assay in C3H/10T 1/2 Mouse Fibroblasts

STUDY IDENTIFICATION: McGlynn-Kreft, A. M., and McCarthy, K. L. Dithane M-45 mammalian cell transformation test. (Unpublished study No. 84R-055 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated November 19, 1984.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 4-3-86

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1. CHEMICAL: Mancozeb; Dithane M-45, coordination product of zinc and manganese ethylene bis-dithiocarbamate.

2. TEST MATERIAL: Dithane M-45, sample TD 83-224, lot No. C842, prepared by Rohm and Haas Co. had a purity of 88 percent; its physical description was not reported.

3. STUDY/ACTION TYPE: Mutagenicity--In vitro transformation assay in C3H/10T 1/2 mouse fibroblasts.

4. STUDY IDENTIFICATION: McGlynn-Kreft, A. M., and McCarthy, K. L. Dithane M-45 mammalian cell transformation test. (Unpublished study No. 84R-055 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated November 19, 1984.) Accession No. 259044.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
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Signature: Nancy E. McCarroll
Date: 4-3-86

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6. APPROVED BY:

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Genetic Toxicology
Technical Quality Control
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Signature: I. Cecil Felkner
Date: 4-3-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irving Mauer
Date: 04-04-86

Jane Harris, Ph.D.
EPA Section Head

Signature: Jane Harris
Date: 04-04-86

7. CONCLUSIONS:

A. Under the conditions of this assay and in the absence of S9 activation, Dithane M-45 at doses of 0.05, 0.15, 0.25, 0.4, and 0.5 µg/mL did not cause an increase in the number of transformed foci in C3H/10T 1/2 cells. The performance of the assay without an exogenous metabolic activation system is an acceptable practice because this cell line can metabolize certain chemicals to active carcinogens; the presence of this metabolic system was adequately demonstrated by the positive response with the known procarcinogen/promutagen, 7,12-dimethylbenzanthracene (DMBA).

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Dithane M-45, sample TD83-224, lot No. 0842, was described as a fungicide with a purity of 88 percent; it was suspended in water. The stock solution and required dilutions were prepared on the day of treatment.

2. Cell Line: Clone 8 of C3H/10T 1/2 mouse embryo fibroblast cells were obtained from Dr. Charles Heidelberger, University of Southern California, Los Angeles. Logarithmic phase cultures, grown from frozen stock cultures, were used as the target cells.

3. Cytotoxicity Assay: Prepared cells, seeded at a density of 200 cells per plate, were exposed in triplicate to an unspecified number of test material concentrations spanning at least a 4-log dose range. After a 24-hour exposure to the test material, the medium was removed and cells were incubated with growth medium for 9-10 days. Surviving colonies were fixed, stained, counted, and compared to the number of colonies in the solvent control.

4. Cell Transformation Assay: Based on the results of the cytotoxicity assay, five doses of test material estimated to yield growth in a survival range of > 90 percent to < 50 percent were selected for the cell transformation assay.

a. Exposure: Prepared cultures, seeded with 200 or 2,000 cells/plate, were treated with the five selected doses of

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[1] Only items appropriate to this DER have been included.

the test material, solvent, or positive control. After a 24-hour exposure, the medium was removed and cultures were refed with fresh growth medium. Cells seeded at the lower density were used to determine the plating efficiency. Surviving colonies in these plates were fixed, stained, and counted after 9-10 days of incubation. The remaining cultures, seeded at 2,000 cells/plate, were periodically refed with growth medium throughout the 6-week incubation period. At the conclusion of incubation, cells in the transformation plates were fixed, stained, and scored for transformed foci.

b. Scoring Transformed Foci: Foci were scored by the criteria of Reznikoff et al.[2] as follows:

Type I--Densely stained areas, composed of tightly packed cells.

Type II--More densely stained areas than Type I, with piling up of cells and overlapping nuclei.

Type III--Highly polar, piling up of cells, composed of areas exhibiting crisscrossing at the interface of the focus and monolayer.

5. Evaluation Criteria: The assay was considered positive if a dose-response relationship was apparent or the incidence of plates with Type III foci at one dose level was significantly higher than the historical untreated and solvent controls.

6. The incidence of plates with Type III foci was statistically compared to the historical untreated and solvent controls by the Fisher Exact test.

7. Evaluation Criteria for Postive Control: Results for the positive control were not analyzed by statistics; the protocol stated that the positive control group must yield an incidence of at least 15 percent of the plates with Type III foci for the positive control to be considered acceptable.

B. Protocol: See Appendix B.

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[2] C. A. Reznikoff, D. W. Brankow, and C. Heidelberger. Establishment and characterization of a cloned cell line C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res. 33(1973): 3231-38.

12. REPORTED RESULTS:

Cytotoxicity Assay: The preliminary cytotoxicity assay was conducted with 14 concentrations of the test material ranging from 0.1 to 1000 µg/mL, two doses of the positive control, DMBA, and the solvent control. Precipitation of Dithane M-45 occurred at all doses above 5 µg/mL, and no cells survived doses ranging from 2.5 to 1,000 µg/mL. Increasing survival (3-81 percent) was observed in a descending dose-related manner for the remaining test concentrations (1.0-0.1 µg/mL). Percent survival for 1.0 and 2.5 µg/mL DMBA, the positive control, was 59 and 56 percent, respectively.

Transformation Assay: Based on the preliminary cytotoxicity findings, doses selected for the transformation assay were 0.05, 0.15, 0.25, 0.4, and 0.5 µg/mL. The concentration of DMBA used was 0.5 µg/mL. Twenty replicates were plated for the five selected doses of the test material and untreated and positive controls; 30 replicates were used for the solvent control. Plating efficiency was determined from the counts of triplicate plates for all test doses and controls. Following exposure, survival ranged from 14 to 96 percent for doses spanning a 0.5 to 0.05 µg/mL concentration range. No Type III foci were found following exposure of the cells to five doses of the test material; statistics were, therefore, not performed. Representative data from this assay are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "This study demonstrates that Dithane M-45 produces no adverse effects in the Mammalian Cell Transformation Test under the conditions specified."

B. A quality assurance statement was signed and dated November 14, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was properly conducted and the authors' interpretation of the data was correct. There was a dose-related increase in cytotoxicity that accompanied exposure to increased doses of the test material. Since no validated exogenous metabolic activation system currently exists for this test, using the C3H/10T 1/2 transformation assay in the absence of S9 activation is acceptable.[a] The sensitivity of the test system to detect the

[a] C. Heidelberger, A. E. Freeman, R. J. Pienta, A. Sivak, J. S. Bertram, B. C. Casto, V. C. Dunkel, M. W. Francis, T. Kakunaga, J. B. Little, and L. Schechtman. Cell transformation by chemical agents--a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 114(1983): 283-385.

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TABLE 1. Representative Results of the C3H/10T 1/2 Transformation Assay with Dithane M-45

Substance	Dose (µg/mL)	% Survival[a]	No. Plates w/Type II Foci/ Total No. Replicates	% Replicates w/Type II Foci	No. Plates w/Type III Foci/ Total No. Replicates	% Replicates w/Type I Foci
Negative Control						
Culture Media	--	99	0/20	0	0/20	0
Solvent Control						
Water	--	100	0/30	0	0/30	0
Positive Control						
7,12-dimethyl-benzanthracene	0.5	78	5/20	25	9/20	45[c]
Test Material						
Dithane M-45	0.5[b]	14	1/20	5	0/20	0

[a] $\frac{\text{No. of colonies with test dose}}{\text{No. of colonies with solvent control}} \times 100$

[b] Highest dose tested; values for transformation assay at lower doses (0.4, 0.25, 0.15, and 0.05 µg/mL) were comparable to the solvent control. Percent survival at these doses ranged from 26 percent at 0.4 µg/mL to 96 percent at 0.05 µg/mL. These data were, therefore, not selected as representative.

[c] Positive by the authors' criterion; ≥ 15% increase in plates with Type III foci.

induction of transformants was adequately demonstrated by the positive control, 0.5 µg/mL DMBA, although this dose was equal to the highest dose of Dithane M-45 assayed. Hence, the assay system appeared to have the appropriate enzymes to metabolize DMBA to a form that is active for inducing cell transformation.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-7; Appendix B, Protocol, CBI pp. 15-22.

APPENDIX A
Materials and Methods

Mancozeb TH Review 005425

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____ Description of quality control procedures.

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005425

EPA: 68-02-4225
DYNAMAC No. 009-A12
April 3, 1986

DATA EVALUATION RECORD

MANCOZEB

Mutagenicity--*In vitro* Transformation Assay for Promotion in C3H/10T 1/2 Mouse Fibroblasts with Ethylenethiourea

STUDY IDENTIFICATION: McLeod, P. L. and Doolittle, D. J. Ethylenethiourea mammalian cell transformation test for promotion. (Unpublished study No. 84R-298 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated May 29, 1985.) Accession No. 259044.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 7-12-86

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1. CHEMICAL: Mancozeb; ethylenethiourea.

2. TEST MATERIAL: Ethylenethiourea prepared by Rohm and Haas, sample TD 83-223, lot No. DB8-36, had a purity of 99.8 percent; its physical description was not reported.

3. STUDY/ACTION TYPE: Mutagenicity--in vitro transformation assay for promotion in C3H/10T 1/2 mouse fibroblasts with ethylenethiourea.

4. STUDY IDENTIFICATION: McLeod, P. L. and Doolittle, D. J. Ethylenethiourea mammalian cell transformation test for promotion. (Unpublished study No. 84R-298 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated May 29, 1985.) Accession No. 259044.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-3-86

Brenda Worthy, M.T.
Independent Reviewer
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Signature: I. Cecil Felkner
Date: 4-3-86

Irving Mauer, Ph.D.
EPA Reviewer

Signature: Irving Mauer
Date: 04-04-86

Jane Harris, Ph.D.
Acting EPA Section Head

Signature: Jane Harris
Date: 04-04-86

7. CONCLUSIONS:

A. Under the conditions of the assay, ethylenethiourea at 333 µg/mL did not cause an increase in the number of transformed foci either in initiated or uninitiated C3H/10T 1/2 cells at a presumed maximum tolerated noncytotoxic dose. However, the assay was conducted with only one test dose, which may not be sufficient to conclude that the test material is not an in vitro tumor promoter.

B. The study is unacceptable because a single dose level is not sufficient for establishing promoter action.

8. RECOMMENDATIONS:

Dr. Craig J. Boreiko,[1] a noted expert on initiation/promotion assays, recommends the use of more than one dose level because promoters frequently induce erratic and nondose-related effects. In lieu of established guidelines for initiation/promotion assays, we feel his suggestions should be considered. We, therefore, recommend that the test material should be assayed at more than one dose or the authors should justify the use of a single treatment level.

Items 9 and 10--see footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Ethylenethiourea, sample TD 83-223, lot No. DB8-36, had a purity of 99.8 percent and was dissolved in dimethylsulfoxide (DMSO).

2. Cell System: Clone 8 of C3H/10T 1/2 mouse embryo fibroblast cells was obtained from Dr. Charles Heidelberger, University of Southern California, Los Angeles. Logarithmic phase cultures, grown from frozen stock cultures, were used as the target cell.

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[1] Boreiko, C. J., Chemical Institute of Toxicology, Research Triangle Park, NC, personal communications.

[2] Only items appropriate to this DER have been included.

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not determined for uninitiated or initiated cultures exposed to the test material, but was determined for selected controls. Surviving colonies on these plates were fixed, stained, and counted after 9-10 days of incubation. Throughout the approximately 6-week promotion phase, cells were continuously refed with media containing the test dose, solvent, or control promoters. At the conclusion of incubation, cells in the transformation plates were fixed, stained, and scored for transformed foci.

b. Scoring Transformed Foci: Foci were scored by the criteria of Reznikoff et al.[a] as follows:

Type I-- Densely stained areas, composed of tightly packed cells.

Type II-- More densely stained areas than Type I with piling up of cells and overlapping nuclei.

Type III-- Highly polar, piling up of cells, composed of areas exhibiting crisscrossing at the interface of the focus and monolayer.

5. Evaluation Criteria: The assay was considered positive if an increase in Type III foci was observed. If an appreciable increase in Type II foci occurred, the test would be repeated with more replicates and/or more or different test concentrations.

6. Evaluation Criteria for Positive Controls: The positive "complete" carcinogen control, DMBA, must yield an incidence of at least 15 percent of the plates with Type III foci to be considered valid evidence of test system sensitivity. Similarly, the promoter control, TPA, must increase the incidence of plates with Type III foci relative to the initiating agent alone for the assay to be considered acceptable. The percent increase was not specified.

7. The data were not statistically analyzed.

B. Protocol: See Appendix B.

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[a] Reznikoff, C. A., Brankow, D. W., and Heidelberger, C. Establishment and characterization of a cloned cell line C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res. 33(1973): 3231-38.

3. Cytotoxicity Assays:

   a. Range-Finding Cytotoxicity Test: Prepared cells, seeded at a density of 200 cells per plate, were exposed in triplicate to three concentrations of the test material.

      Cells were exposed either for 24 hours or continuously throughout a 9-day incubation period. After the 24-hour exposure, the medium was removed and cells were incubated with growth medium for 8 days. Surviving colonies for both exposures were fixed, stained, and counted.

   b. Cytotoxicity Assay with the Initiating Agent: Cultures, seeded at a density of 2000 cells per plate, were exposed to the initiating agent, 0.5 µg/mL N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), for 4 hours. Five and 9 days after initiation, cell viability was determined from three control cultures. The remaining cultures were continuously refed with media containing the three selected test doses or the solvent control; viability was monitored daily for 4 days and on alternate days thereafter until termination of the cytotoxicity test, day 11 after initial treatment with the test compound. A growth curve was plotted (total number of cells vs. days post-treatment) to determine growth inhibition.

4. Cell Transformation Assay for Promotion: Based on the combined results of the cytotoxicity assays, a single test dose was selected for the cell transformation test with promotion.

   a. Exposure: The appropriate number of prepared cultures, seeded with either 200 or 2000 cells/plate, were treated with the following agents: 0.5 µg/mL MNNG, 0.5 percent acetone (MNNG solvent), 0.5 µg/mL 7,12-dimethylbenzanthracene (DMBA), or 1.0 µg/mL 3-methylcholanthrene (MCA). Exposure to the initiating agents, MNNG or acetone, was terminated at 4 hours; the exposure period for DMBA or MCA was 24 hours. Five days after dosing, initiated cultures were refed with media containing the selected dose of the material being assayed for promoter activity, the solvent controls, DMSO or acetone, or the known promoting agent, 0.25 µg/mL 12-o-tetradecanoylphorbol-13-acetate (TPA); this treatment was continued for 6 weeks (promotion phase). Therefore, a single dose of the test material was exposed to 20 replicates of untreated cells, 20 replicates of MNNG-initiated cells, or 20 replicates of acetone-treated cells. The remaining control groups, DMSO or TPA, were similarly added to 20 replicate untreated or preinitiated cultures. Cultures treated with DMBA or MCA were not exposed to promoting agents because these compounds do not require promoter action to transform cell cultures. Plating efficiency was

12. REPORTED RESULTS:

Cytotoxicity Assays:

a. Range-Finding Cytotoxicity Test: The preliminary cytotoxicity assays were conducted with 100, 333, and 1000 µg/mL of the test material. Following a 24-hour and a 9-day continuous exposure, cytotoxicity was not evident at any dose tested.

b. Cytotoxicity Assay with Initiating Agent: Cultures preinitiated with 0.5 µg/mL MNNG were continuously exposed to 100, 333, or 1000 µg/mL of the test material. Based on the reported growth curve results, a 60 percent inhibition of cells was plotted at day 4 for the highest dose. By day 7, cell growth at this level was 22 percent less than the solvent control. At 333 µg/mL, growth inhibition (30 percent) was plotted for day 4; however, by day 7 cells exposed to 333 µg/mL recovered and exceeded the growth in the solvent control group. Throughout the remaining incubation period, cell growth at this test level consistently exceeded growth in the solvent control. Based on these findings, 333 µg/mL was selected for the transformation assay for promotion.

c. Transformation Assay with Promotion: The selected dose, 333 µg/mL, was continuously applied to untreated and acetone-/ or MNNG-pretreated cells. No foci were observed on untreated or acetone-treated test-dose-promoted plates. Fifteen percent of the plates containing cells initiated with MNNG, promoted with 333 µg/mL, had Type II foci; no Type III foci were scored. Representative results are shown in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "This study demonstrates that ethylenethiourea does not promote morphological transformation in the Mammalian Cell Transformation Test for Promotion under the conditions specified."

B. A quality assurance statement was signed and dated March 29, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that a properly designed study was conducted, and the authors' interpretation of the data was correct. No *in vitro* assays to detect tumor promoters have been validated and probably must await the clearer understanding of tumor promotion mechanisms before their use in screening programs can be fully realized.

TABLE 1. Representative Results of the C3H/10T 1/2 Transformation Assay for Promotion with Ethylenethiourea (ETU)

Substance[a]		No. Plates w/ Type II Foci/ Total No. Replicates	% Replicates with Type II Foci	No. Plates w/ Type III Foci/ Total No. Replicates	% Replicates with Type III Foci
Initiator	Promoter				
Negative Control					
Media	Media	0/20	0	0/20	0
Solvent Control					
Media	DMSO (0.33%)	0/20	0	0/20	0
Acetone (0.5%)	DMSO (0.33%)	0/20	0	0/20	0
MNNG (0.5 µg/mL)	DMSO (0.33%)	2/20	10	0/20	0
Positive Control					
DMBA (0.5 µg/mL)	—	6/20	30	8/20	40[b]
MCA (1.0 µg/mL)	—	12/20	60	10/20	50[b]
MNNG (0.5 µg/mL)	TPA (0.25 µg/mL)	11/20	55	9/20	45[c]
Test Substance					
Media	ETU (333 µg/mL)	0/20	0	0/20	0
Acetone (0.5%)	ETU (333 µg/mL)	0/20	0	0/20	0
MNNG (0.5 µg/mL)	ETU (333 µg/mL)	3/20	15	0/20	0

[a] DMSO = Dimethylsulfoxide
MNNG = N-methyl-N'-nitro-N-nitrosoguanidine
TPA = 12-o-Tetradecanoyl-phorbol-13-acetate
DMBA = 7,12-Dimethylbenzanthracene
MCA = 3-Methylcholanthrene

[b] Positive by the authors' criterion (≥ 15% increase in plates with Type III foci).

[c] Positive by the authors' criterion (increased incidence of plates with Type III foci as compared to initiating agents alone).

The authors stated, in accordance with Frazelle et al.[4], that "a non-toxic concentration of test compound is the maximum tolerated dose for assessing promoting activity in this assay."

We confirmed this statement with Frazelle and Boreiko[5] who indicated that the majority of promoters are noncytotoxic. Boreiko[6] recommended, however, that more than a single dose should be assayed (five doses of an unknown agent are routinely evaluated in his laboratory) since promoters frequently induce erratic and nondose-related effects. Although ethylenethiourea was negative at the selected concentration, it is possible that 333 µg/mL was not the effective level and tumor promotion could have been detected if more doses were evaluated.

No established guidelines exist for initiation and promotion assays. Since Dr. Boreiko is a recognized expert in this area, his recommendation should be considered appropriate in lieu of published guidelines. It is our assessment, therefore, that the results reported by the authors are insufficient to support the conclusion that ethylenethiourea does not promote neoplastic transformation in this assay.

The ability of the known tumor promoter, TPA (0.25 µg/mL), to induce neoplastic transformation in initiated cells was demonstrated. Similarly, the direct induction of transformants by DMBA (0.5 µg/mL) and MCA (1.0 µg/mL) was adequately shown. Although no criteria were presented to evaluate a positive effect with MCA, we assumed that the criterion reported for DMBA (≥ 15% increase in plates with Type III foci) applied to both polycyclic aromatic hydrocarbons.

Item 15—see footnote 2.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-8, page 5 missing; Appendix B, Protocol, CBI pp. 17-30.

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[4] Frazelle, J. H., Abernethy, D. J., and Boreiko, C. J. Determination of cell culture conditions optimal for the study of initiation and promotion in C3H 10T 1/2 cells. Environmental Mutagenesis 4(1982): 331-332.

[5] Sanchez, J. H. (nee Frazelle) and Boreiko, C. J., Chemical Institute of Toxicology, Research Triangle Park, NC, personal communications.

[6] Ibid.

APPENDIX A

Materials and Methods