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

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2/24/86

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA File Symbol 33677-R
Methylene Bisthiocyanate (MBT) for
Manufacturing Use
Accession No. 259007

Caswell No. 565

FROM: William Woodrow, Ph.D. WSW 2-18-86
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: A.E. Castillo, PM 32
Disinfectants Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D. ABK 2/19/86
Supervisory Pharmacologist, Section VII
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Tenneco Organics, Ltd.
Rockingham Works
Avonmouth
Bristol
United Kingdom BS11 OYT

Action Requested:

Tenneco submitted toxicity studies to the Agency for evaluation that are required to support Methylene Bisthiocyanate (MBT) for Manufacturing Use only. Tenneco states that the product would be supplied to formulators of industrial microbiocides and preservatives.

Recommendations:

1. The toxicity studies submitted by Tenneco, Ltd. are acceptable and support a Manufacturing Use type product.

2. The toxicity data were evaluated as follows:

   a. Acute Oral LD_{50}, rat: 99 percent pure MBT.

LD_{50} = 104.9 (73.0 to 146.7 - 95% C.L.) mg/kg
slope = 3.2
Toxicity Category: II
Classification: Core-Minimum Data

b. Acute Dermal LD_{50}, rat: 95 percent pure MBT.

LD_{50} > 2 grams/kg bwt
Toxicity Category: III
Classification: Core-Minimum Data

c. Primary Ocular Irritation, rabbit:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether. A 5 percent solution (5mL + 95 mL water) was tested.

Ocular PI score = 52.7, a severe ocular irritant.
Toxicity Category: I

Classification: Core-Minimum Data
(The pesticide guidelines state that solids should not be diluted; however, since the severe nature of MBT was determined, the study was classified Minimum.)

d. Primary Dermal Irritation, rabbit:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether.

Dermal PI = 6.42, a severe skin irritant.
Toxicity Category: I
Classification: Core-Minimum Data

e. Primary Ocular Irritation, rabbit:

0.5 percent aqueous solution of 10 percent MBT in ethylene glycol mono methyl ether.

Ocular PI score = 13.5, mild to moderate irritant.
Toxicity Category: III
Classification: Supplementary Data
(Test material was excessively diluted.)

f. Acute Dermal LD_{50}, rat:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether

Dermal LD_{50} = 3.679 (2.388 to 5.667 95% C.L.) mL/kg.
Toxicity Category: III

Classification: Supplementary Data
(Test material should have been moistened only prior to testing.)

g. Primary Dermal Irritation, rabbit:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether.

Dermal P.I. = 6.5, a severe skin irritant.
Toxicity Category: I
Classification: Core-Minimum Data
(Should have used 24-hour exposure. Since severe effects were determined in the 4-hour exposure used, classified Minimum.)

h. Primary Dermal Irritation, rabbit:

Five percent aqueous solution of 10 percent Methylene Bisthiocyanate in ethylene glycol mono methyl ether.

Dermal P.I. score = 3.42, a moderate irritant.
Toxicity Category: II
Classification: Supplementary Data
(Test material should have been moistened only.)

i. Primary Dermal Irritation, rabbit:

0.5 percent aqueous solution of Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether.

Dermal P.I. score = 0.25
Toxicity Category: IV
Classification: Supplementary Data
(Test material should have been moistened only.)

j. Dermal Sensitization, guinea pig:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether, was a moderate sensitizing agent.
Classification: Core-Minimum Data

k. Ames Metabolic Activation Study to Assess Genetic Potential of Methylene Bisthiocyanate:

MBT dissolved in DMSO.
Methylene Bisthiocyanate did not show mutagenic potential in the Ames study.
Classification: Core-Minimum Data

1. Micronucleus Study in the Mouse to assess MBT genetic potential:

One percent MBT dissolved in methylcellulose--dilutions made in water.

LD_{50} used to calculate dose levels:
44.72 (36.53 to 54.75 - 95% C.L.) mg/kg

Three doses used in micronucleus study:
1) 20 percent of LD_{50}, 2) 40 percent of LD_{50}, and 3) 80 percent of LD_{50}
(8.94, 17.89, and 35.78 mg/kg x 2, respectively).

Results - MBT did not show any mutagenic potential in the mouse micronucleus study.
Classification: Core-Minimum Data

3. The label signal word and precautionary statements appear to be satisfactory. (See p. 20 of this review)

4. No additional toxicity studies are necessary to support a Manufacturing Use designation.

Review of Data

1. Acute Oral LD_{50}, rat:

Sponsor: Albright & Wilson Ltd. Tenneco Organics Division, Bristol, England.
Tester: Food and Drug Labs, Waverly Division, NY #6914A, May 6, 1981.

Test Material:

Methylene Bisthiocyanate sample #4251, powder
99 percent pure.

Five male and five female Sprague-Dawley rats per group, treated by gavage on one-time basis: All animals observed for 15 days for gross toxic signs, body weights recorded initially and at termination. Animals dying were subjected to gross necropsies.

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Results:

Acute Oral LD_{50}

Group	Dose (mg/kg)	Dead/Total
1	40.0	1/10
2	71.0	3/10
3	126.0	6/10
4	225.0	8/10
5	400.0	10/10

Necropsies revealed scattered blood-like viscous liquid in intestines at each dose level; in one to three individuals per dose level.

Acute oral LD_{50} (mg/kg) = 104.9
95% C.L. = 73.0 to 146.7 mg/kg, slope = 3.2

Reference (calculations): Finney, D.J., Jr. Statistical Methods in Biological Assay 2nd Edition. London: Griffin Press, 1971.
Toxicity Category: II
Classification: Core-Minimum Data

2. OECD Acute Dermal Toxicity, rat:

Sponsor: Albright & Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#697/8404, May 14, 1984.

Test Material:

Methylene Bisthiocyanate 95 percent pure sample #3478 moistened with arachis oil B.P.

Following lab acclimation, 5 male and 5 female Sprague-Dawley rats were each treated with 2000 mg/kg of MBT by applying doses to 6 x 3 cm, clipped skin sites that had been premoistened with arachis oil. Treated sites were covered with gauze, elastic adhesive backed with aluminum foil around the trunk. Twenty-four hours later, dressings were removed, treated skin and surrounding hair were sponged with warm water and dried. Observations were made 1/2, 1, 2, 3, 4, and 5 hours, and once per day thereafter, for 14 days following treatment for mortality and toxic effects. Body weights recorded on days 0, 7, and 14. All dying and surviving animals (day 14) were necropsied.

Results:

All animals survived. No toxic effects were observed. Slightly depressed body weights recorded during first week of observation. No gross abnormalities at necropsy.

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Acute dermal LD_{50} (MBT) > 2 grams/kg bwt
Toxicity Category: III
Classification: Core-Minimum Data

3. Primary Ocular Irritation, rabbit:

Sponsor: Albright & Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#360/903, March 1979.

Test Material:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether. For this test a 5 percent aqueous solution/suspension (5 mL + 95 mL of water) was used.

One-tenth mL of test material was instilled into the conjunctival sac of each right eye of 6 NZW rabbits. Upper and lower lids held together 4 seconds after application. Untreated eyes served as controls.

Eyes were examined 1, 2, 3, 4, and 7 days posttreatment, according to Draize.

Results:

All rabbits showed discomfort immediately following instillation. Five minutes following instillation, the nictitating membrane was swollen in all rabbit eyes.

The test material caused severe ocular irritation; conjunctival reddening, chemosis and discharge, corneal opacity and iridial congestion and/or hemorrhage. Corneal damage persisted to day 7 in four of six rabbits.

Ocular PI score = 52.7
Toxicity Category: I
Classification: Core-Minimum Data
(Subpart F testing protocol states--do not moisten powder (or dilute); however, damage at much diluted state did elicit ocular nature of MBT).

4. Primary Dermal Irritation, rabbit:

Sponsor: Albright & Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#411/901, January 1979.

Test Material:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether--no further dilution.

Prior to treatment, the dorsal flanks of six male NZW rabbits were clipped. On day of test, the right side of each rabbit (skin) was abraded, left sides remained intact. 0.5 mL of test material was placed under patches on intact and abraded sites on each animal. Patches were held in place by adhesive tape, followed by covering with rabbit "corsets" for each animal.

Results:

All dressings were removed 24 hours after dermal applications, and the treated sites were scored at 24 and 72 hours according to Draize. Severe irritation showed in 4/6 rabbits at both intact and abraded test sites. At 24 hours severe edema and tissue necrosis occurred with erythema and edema extending beyond the test site. At 72 hours eschar formation was visible on three animals.

Dermal PI index = 6.42, a severe skin irritant.
Toxicity Category: I
Classification: Core-Minimum Data

5. Primary Ocular Irritation, rabbit:

Sponsor: Albright & Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#49/902, February 1979.

Test Material:

Methylene Bisthiocyanate

A 0.5% aqueous solution of MBT 10 percent in ethylene glycol mono methyl ether. (A total dilution of 0.05.)

One tenth mL of the diluted test material was instilled into right eyes of six NZW rabbits, treated eye lids were held shut 4 seconds after application. Untreated eyes served as controls.

Treated eyes were scored according to Draize at 1, 2, 3, 4, and 7 days posttreatment.

Results:

Approximately 3 hours following treatment the nictitating membrane in all treated eyes were swollen. This effect

disappeared by 24 hours. Eye irritation was mainly confined to the conjunctivae. All rabbits showed reddening of the palpebra, chemosis and/or lacrimation. All effects disappeared by day 7 in three rabbits; minimal erythema remained in three animals. Slight corneal opacity in three rabbits on days 1 and 2. No corneal opacity by day 3.

PI ocular score = 13.5, mild to moderate irritant.
Toxicity Category: III
Classification: Supplementary Data
(Test material too dilute, only 0.05%.)

6. Acute Dermal LD_{50}, rat:

Sponsor: Albright and Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#80/901, January 1979

Test Material:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether.

The hair at dorsal, lateral, and ventral areas of 16 female Wistar rats was removed by clipping. The rats were treated by spreading dosages evenly over clipped skin and covering treated sites with aluminum-backed adhesive plaster as follows:

Groups No. Rats	Dosage (mL/kg)
4	2.0
4	2.71
4	3.68
4	5.00

All treatment site dressings were removed after 24 hours, exposure sites were sponged with warm water and dried.

Results:

Dose (mL/kg)	Dead/Total
Mortality	
2.00	1/4
2.71	0/4
3.68	3/4
5.00	3/4

Animals were observed for 7 days. The acute dermal LD_{50} was calculated using the moving average of Thompson. (Thompson, W.R. (1947) Bact. Rev. (11) 115-145.)

Dermal LD_{50} for MBT 10 percent in ethylene glycol mono methyl ether was:

3.679 (2.388--5.667 95% C.L.) mL/kg

Toxicity Category: III
Classification: Supplementary Data
(The test material-dry powder-should have been moistened only, prior to application.)

7. Primary Dermal Irritation, rabbit:

(4-hr exposure)
Sponsor: Albright and Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#793/8103, July 7, 1981.

Test Material: 10 percent Methylene Bisthiocyanate in ethylene glycol mono methyl ether.

One mL of test liquid was placed under 2.5 cm sq absorbent lint after clipping the dorsal/flank areas of 8 NZW rabbits free of hair; 1 mL test material to one treatment site on both sides of each animal. Absorbent lint patches were covered by 2.5 cm sq polyethylene patches, which in turn were held in place by a backing with 4 cm sq lint patches. These materials were all covered with Sleek adhesive wrapping and an elasticated corset. Exposure time was 4 hours, after which wrappings were removed and treated sites were sponged with warm water.

Results:

Animals were observed at 24 and 72 hours after treating and irritation was scored according to Draize.

Severe cutaneous irritation was produced in all test animals.

By 7 days posttreatment, eschar formation had developed in one rabbit, erythema had subsided in another rabbit, and erythema became less marked in two other animals. Erythema persisted in the remaining animals.

Dermal P.I. score = 6.5, a severe skin irritant.
Toxicity Category: I
Classification: Core-Minimum Data
(A 24-hour exposure should have been used but since severe effects resulted in this 4-hr exposure, a Minimum grade was justified).

8. Primary Dermal Irritation, rabbit:

Sponsor: Albright and Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#258/903, March, 1979.

Test Material:

A 5 percent aqueous solution of 10 percent Methylene Bisthiocyanate in ethylene glycol mono methyl ether.

Five-tenths ml of test material was placed under lint patches backed with polyethylene at one abraded and one intact skin site on each of six male NZW rabbits. Hair at test sites was removed 1 day prior to application by clipping. Sleek adhesive tape held the treatment patches in place, and rabbit corsets were finally installed over the Sleek tape.

All dressings were removed after 24 hours exposure, and treated sites were scored according to Draize at 24 and 72 hours postexposure.

Results:

Well-defined erythema at intact and abraded sites at 24 and 72 hours in 5/6 animals. Slight eschar formation in two animals at 72 hours. Slight to moderate edema at 24 hours at all test sites on all animals. These effects (edema) disappeared in 2 of 6 animals at 72 hours.

Dermal P.I. score = 3.42, a moderate irritant.
Toxicity Category: II
Classification: Supplementary Data
(Test material should have been moistened only--not diluted--prior to testing.)

9. Primary Dermal Irritation, rabbit:

Sponsor: Albright and Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#412/901 January 1979.

Test Material: 0.5 percent aqueous solution of Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether.

Six NZW rabbits received 0.5 mL test material at each of one intact and one abraded skin test sites that had been previously clipped free of hair.

Test sites were protected by occlusive wrappings, and at 24 hours wrappings were removed.

Results:

Test sites were scored for irritation at 24 and 72 hours posttreatment.

Dermal P.I. score = 0.25
Toxicity Category: IV
Classification: Supplementary Data
(The test material should have been moistened only, prior to application.)

10. Dermal Sensitization, guinea pig:

Sponsor: Albright and Wilson, Ltd.
Tester: Safepharm Labs, Ltd., Derby, England.
#112/901, March 1979.

Test Material:

Methylene Bisthiocyanate 10 percent in ethylene glycol mono methyl ether.

Three pairs of intradermal injections were given to each of 10 Dunkin/Hartley female guinea pigs, at 0.1 mL per injection, at a 4 by 6 cm area on guinea pig shoulders previously clipped free of hair:

Injection sites (two each): Initial Induction

a. 0.1 mL of Freund's complete adjuvant.

b. 0.1 mL of a 5 percent solution of the test material alone.

c. 0.1 mL of a 5 percent solution of test material emulsified in Freund's adjuvant.

One week after the initial induction injection, 2- by 4-inch patches of Whatman's filter paper saturated with a 10 percent aqueous solution of MBT was applied to each of

the injection induction sites. The Whatman paper patches were covered by overlapping impermeable adhesive bandages, and finally covered by an elastic bandage wrapped around the guinea pig torso. These second induction topical exposures were left in place for 48 hours.

A further group of untreated animals (four) served as controls.

Two weeks following the second induction treatment, animals were challenged. Hair was removed from a 5 by 5 cm flank area by clipping and shaving. Five percent aqueous test solution (MBT) was applied to saturation to 2 by 2 cm patches of Whatman No. 4 filter paper, which were applied to the prepared sites. Challenge treatment paper was held in place under a 4 cm strip of 2.5 cm Sleek adhesive, which was then firmly secured with Elastoplast elastic adhesive wrapped around the trunk. The test and four control animals were all challenged.

Results:

Challenge sites were evaluated 24 and 48 hours after patch removal (following 24 hours challenge exposure), and scores prepared by comparing test sites with controls.

Five of 10 test animals showed more erythema than control animals, thus, the test material produced a 50 percent sensitization rate.

Under the conditions of the present experiment, 10 percent MBT was considered a moderate sensitizing agent.

Classification: Core-Minimum Data

11. Ames Metabolic Activation Study to Assess Genetic Potential of Methylene Bisthiocyanate:

Sponsor: Albright and Wilson, Ltd.
Tester: Huntingdon Research Center, England.
(No Study No., No Date)

Test Material:

Methylene Bisthiocyanate dissolved in DMSO
DMSO - negative control

Neutral Red	pos. control
2-acetylamino fluorene	pos. control
2-amino anthracene	pos. control

Bacteriostatic study--Determine choice of maximum concentration of MET to employ; 0.1 mL aliquots of bacterial suspensions added to histidine deficient medium, which was overlayed onto minimal agar. Wells were then cut into the agar and 0.001 to 10,000 ug/well were added.

Mutation study--Histidine-deficient Salmonella typhimurium test strains:

S. typhimurium TA-1535--detect base-pair substitution mutagens.

S. typhimurium TA-1537--detect frame-shift mutagens.

S. typhimurium TA-1538--detect frame-shift mutagens.

S. typhimurium TA-98--sensitive to weaker frame-shift mutagens.

S. typhimurium TA-100--detect base-pair substitution mutagens.

One-tenth mL aliquots of test material for each dilution tested, the DMSO negative control, and each of the positive controls were placed in separate duplicate sets of bijou bottles.

One-tenth mL aliquots of standardized bacterial suspensions were added to the bottle sets for each of the bacterial strains tested.

An S-9 liver microsome mix at 0.5 mL was added to each bottle for one of the duplicate sets for each bacterial strain; the other set of bottles received 0.5 mL of saline. 2.8 mL of histidine deficient agar was added to each of the bottles, mixed, and then overlayed onto 15 mL of minimal agar. Triplicate plates were used for each test material concentration.

Plates were incubated at 37 °C for 72 hours and the number of revertants to histidine prototrophy per plate was counted.

Results:

Bacteriostatic study--A first bacteriostatic test produced inhibition to all test bacterial strains employed. A second test showed that only the lower dose levels could

be used for the mutation study; these concentrations produced zones of inhibition of 10 to occasionally 13 mm for each of the test organisms:

µg/well
1.0
0.1
0.01
0.001
0.00

MBT revertant colonies:

TABLE I

Strain S. typhimurium	Compound	Concentration of MBT µg/plate	Metabolic Activation	Mean Revertant Colony Counts
TA 98	MBT	5	–	NL
		0.5	–	IL
		0.05	–	83
		0.005	–	88
	DMSO	0	–	86
	MBT	5	+	IL
		0.5	+	85
		0.05	+	89
		0.005	+	96
	DMSO	0	+	89
TA 100	MBT	5	–	NL
		0.5	–	IL
		0.05	–	103
		0.005	–	109
	DMSO	0	–	109
	MBT	5	+	IL
		0.5	+	102
		0.05	+	108
		0.005	+	113
	DMSO	0	+	107
TA 1535	MBT	5	–	NL
		0.5	–	22
		0.05	–	20
		0 005	–	21
	DMSO	0	–	19
	MBT	5	+	IL
		0.5	+	21
		0.05	+	21
		0.005	+	21
	DMSO	0	+	21
TA 1537	MBT	5	–	NL
		0.5	–	7
		0.05	–	7
		0.005	–	8
	DMSO	0	–	7
	MBT	5	+	IL
		0.5	+	8
		0.05	+	7
		0.005	+	7
	DMSO	0	+	8

TABLE I (cont'd.)

Strain S. typhimurium	Compound	Concentration of MBT µg/plate	Metabolic Activation*	Mean Revertant Colony Counts
TA 1538	MBT	5	-	NL
		0.5	-	20
		0.05	-	19
		0.005	-	20
	DMSO	0	-	21
	MBT	5	+	IL
		0.5	+	22
		0.05	+	21
		0.005	+	25
	DMSO	0	+	21

*- = absence
+ = presence
NL = no bacterial lawn
IL = incomplete bacterial lawn

The positive control tests all showed significantly increased numbers of histidine revertants.

Viability tests with each of the test strains on deficient (no histidine) and complete media showed deficient strains to be adequate for testing.

Conclusions:

Table I indicates that MBT was not mutagenic, as presently tested.

Classification: Acceptable Study ~~Core Minimum Data~~

12. Micronucleus Study in the Mouse with Methylene Bisthiocyanate:

Sponsor: Albright and Wilson, Ltd.

Tester: Safepharm Labs, Ltd., Derby, England. #119/8103, March 25, 1981.

Test Material:

Solid, Methylene Bisthiocyanate. For the test, a 1 percent solution of MBT in methylcellulose by dilution in distilled water.

Cyclophosphamide was used as a positive mutagen. Solutions of cyclophosphamide were prepared in distilled water.

A 1 percent solution of methylcellulose in distilled water was used as a negative control.

Range Finder Study--Prior to LD_{50} Study

LACA mice (one male and one female) were separately dosed with 20.0, 50.0, 100.0, or 200.0 mL/kg per group of two animals each per os. Treated animals were observed for 48 hours for mortality.

LD_{50} Determination:

Based on mortality occurring in the range-finding study, log-spaced dose levels were calculated and the following LD_{50} oral dosing schedule was prepared:

Mice (5M/5F)	Dose Level mg/kg	Test Concentration mg/mL	Dose Volume mL/kg
10	20.0	10.0	2.0
10	32.4	10.0	3.42
10	58.5	10.0	5.85
10	100.0	10.0	10.0

Treated animals were observed for 48 hours and mortalities recorded.

Thompsons moving average interpolation method was used to calculate the LD_{50}, based on 48 hours mortality.

[Thompson, W.R. 1947 Bact. Rev. (11) 115-145, using tables computed by Weil (Weil, C.S. 1952 Biometrics (8) 249-262)].

The LD_{50} was then used to calculate dose levels to be used in the micronucleus study:

Low dose:	20% of the LD_{50}
Intermediate dose:	40% of the LD_{50}
High dose:	80% of the LD_{50}

The following dosing schedule for the micronucleus test was employed; animals were treated by the per os route:

TABLE I

Treatment	Animals M	Animals F	Dose Level	Concentration mg/mL	Dose Volume mL/kg
Methyl cellulose (-cont.)	5	5	-	-	10.0 x 2
Methyl Bisthiocyanate Low	5	5	8.94 x 2	5.0	1.79
MBT Intermediate	5	5	17.89 x 2	5.0	3.58
MBT High	5	5	35.78 x 2	5.0	7.16
Cyclophos-phamide (+ control)	5	5	50.0 x 2	5.0	10.0

The MBT animals (three groups) were treated with two single doses of test material, one dose at 0 hours, and one dose at 24 hours, according to the dosing schedule in table 1 above.

The negative and positive control animals were treated with a single dose, as shown in table 1.

Six to 9 hours following the second (MBT) dose, animals were killed by cervical dislocation and bone marrow smears were prepared from femurs. The proximal end of the femur was shortened until a small opening appeared. An Eppendorf centrifuge tube was filled with approximately 1 mL fetal calf serum, and approximately 0.2 mL of serum was withdrawn from the centrifuge tube to a syringe, and bone marrow was aspirated into the syringe. The same procedure was carried out from the distal end of the bone. Both femurs from each animal were similarly aspirated (bone marrow), into the same centrifuge tube using the same aliquot of serum.

Tubes containing aspirated bone marrow were centrifuged at 1000 rpm for 5 minutes. Supernatant fluids were removed by aspiration, bone marrow cell suspensions were transferred to slides, and smears were prepared. Smears

were dried for 24 hours (3/animal), fixed with absolute methanol 5 minutes, stained 10 minutes with Giemsa stain. Smears were cleared after drying with xylene and mounted in Canada balsam.

One thousand polychromatic erythrocytes (PCE) from each animal were examined at 1000X magnification and the number having micronuclei were recorded.

The group mean number of PCE with micronuclei occurring in the low, intermediate, and high dose groups and the positive control group were compared with the group mean number of PCE occurring in the vehicle control group. Statistical analyses were employed where necessary.

Results:

LD_{50}:

44.72 (36.53 to 54.75) mg/kg

Calculated dose levels: (for MBT)

Low dose (20% of LD_{50}) = 8.94 mg/kg x 2
Intermediate dose (40% of LD_{50}) = 17.89 mg/kg x 2
High dose (80% of LD_{50}) = 35.78 mg/kg x 2

Micronucleus study results:

TABLE 2

Group	No. of micronucleated cells in 1000 polychromatic erythrocytes Group Mean	± SE
Vehicle control	2.5	0.582
MBT (Low)	2.56	0.580
MBT (Intermediate)	2.2	0.416
MBT (High)	1.78	0.401
Cyclophosphamide + control	38.8*	2.867

*Significantly larger than the vehicle control group.

Conclusions:

Table 2 shows no significant increase in the number of micronucleated polychromate erythrocytes occurred in MBT treated mice, when compared to the vehicle control.

The positive control group (cyclophosphamide) did show a significant increase in the number of micronucleated polychromatic erythrocytes.

Thus, MBT did not show mutagenic potential in the present Micronucleus Test.

Classification: Acceptable Study ~~Core Minimum Data~~

The registrant should assure himself that the instructions to induce vomiting are appropriate in light of the fact that the product is considered a corrosive.

The registrant should also assure himself that the statement of practical treatment with regard to vomiting is not in conflict with the note to the physician in light of the fact that the product is considered a corrosive.

The following statement needs to be added to the label — This product may cause sensitization.