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

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JUN 10 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Methylene bis (thiocyanate)

Project No: 9-1436
Tox. Chem. No.: 565
BARCODE No.: 245141
EPA ID No: 1448-80

TO: John Lee, PM # 31
Registration Division (H7505C)

THRU: Roger Gardner, Section Head
Review Section 1
Toxicology Branch Roger Gardner 2/13/91 5/31/91
Health Effects Division (H7509C)

FROM: Nguyen Bich Thoa, Ph.D NBThoa
Review Section 1
Toxicology Branch I
Health Effects Division (H7509C)

Registrant: Buckman Labs. International
Musselburgh, Scotland, UK.

ACTIONS REQUESTED:

Review of a metabolism study (85-1) entitled: The Metabolism of Methylene bis (thiocyanate) in the Rat (MRID 410885-01).

CONCLUSIONS:

The disposition and metabolic profile of ^{14}C-Methylene bis (thiocyanate) (^{14}C-MBT) was studied after oral gavage administration to Sprague Dawley rats of both sexes. Rats were dosed with single doses of 3 or 30 mg/kg or with repeated doses of 3mg/kg/day. The results showed that ^{14}C-MBT was rapidly absorbed, extensively metabolized, and rapidly excreted. Over a 4-day period, 94.87 to 99.95% of the dose administered was excreted (63-71% in the urine, 14-19% in the feces, and 11-14% as $^{14}CO_2$ in the expired air). Less than 1% of the administered dose remained in the tissues. A number of unidentified radioactive products were isolated by thin layer chromatography in the urine, feces, plasma, and tissues (liver and kidney). Some unchanged

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test material was recovered in the fecal extracts of rats treated with 30 mg/kg. This study does not completely satisfy the toxicological data requirements of guidelines 85-1 (Metabolism) because the registrant failed to identify the metabolites found in the urine, feces, and tissues. In addition, the registrant is requested to provide information on the purity and composition of the unlabeled test material used in the study and to provide toxicological data validating the use of 30 mg/kg as the high dose. Until all 3 above requirements are satisfied, this study is classified supplementary.

A DER on the above referenced study is attached.

008404

Primary Reviewer: Nguyen B. Thoa, Ph.D 3/5/91
Section 1, Toxicology Branch I (H7509C)
Secondary Reviewer: Roger Gardner 5/31/91
Section 1, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism - Rat (85-1)

Tox Chem No: 565
MRID No: 410885-01
HED Project No: 9-1436

TEST MATERIAL: Methylene bis (thiocyanate)

SYNONYMS: MBT®

SPONSOR: Buckman Labs. International Inc.
Memphis, TN, USA

TESTING FACILITY: Inveresk Research International
Musselburgh, Scotland, UK

STUDY NO.: 137336

REPORT TITLE: The Metabolism of Methylene bis (thiocyanate) in the Rat

AUTHORS: L. Brown, B.D.Cameron, P.J. Mutch, and G. Scott

REPORT ISSUED: July, 1988

CONCLUSIONS: The disposition and metabolism of ^{14}C-methylene bis (thiocyanate) (^{14}C-MBT) was studied after oral (gavage) administration to rats. Male and female rats were dosed with ^{14}C-MBT at single oral doses of 3 and 30 mg/kg and at repeated doses (14 daily doses) of 3mg/kg. ^{14}C-MBT was rapidly absorbed, extensively metabolized, and rapidly excreted. Over a 4-day period, most (94.87-99.95%) of the test compound administered was excreted from the animals. The radioactivity recovered in the urine, feces, and CO_2 in the exhaled air was 63-71, 14-19 and 11-14 percent of the administered dose, respectively. Peak plasma concentrations of radioactivity occurred 1 to 2 hours after the administration of the test compound. A number of radioactive components (none of which co-chromatographed with ^{14}C-MBT) were observed in the urine. Radiolabeled components found in fecal extracts from rats treated with a LD did not co-chromatograph with ^{14}C-MBT but one component found in fecal extracts from rats treated with a HL did. None of the unidentified radioactive components were characterized.

CLASSIFICATION (Core-Grade): The toxicological data provided on the absorption, distribution, and excretion of total radioactivity following single or repeated oral dosings with ^{14}C-MBT is

acceptable. The data provided on the identification of the metabolites of MBT in the urine, feces, expired air, and tissues is incomplete. Guidelines 85-1 requires a characterization of those metabolites. In this study, the only characterizations done were those of $^{14}CO_2$ in the expired air and of the unchanged MBT in the fecal extracts of group Ib rats. In addition the registrant should provide information on the purity and composition of the unlabeled test material used in this report. The purity of the test material was left unstated in the report. Last, according to guidelines 85-1 the HD should produce some toxic/pharmacologic signs. The registrant should state which adverse effect was observed which validated the use of 30 mg/kg as the HD in this study. This study is presently classified supplementary but could be upgraded to acceptable upon satisfaction of the above underlined requirements.

I. MATERIALS:

A. TEST MATERIALS.

The following information was provided for the test compounds used in the study:

	Unlabeled MBT	^{14}C-MBT
Description	Not described	Crystals, 0-5° C storage
Purity %	Not stated	98
Solvent	PEG 400	PEG 400
Source	batch # 7-8046 Buckman Labs.	Lot No. 097F9207 Sigma Chem. Co.
Spec. Activity	N/A	13.1 mCi/mmol (100.6 uCi/mg)
Position of label (*)	N/A	NC-S-(*)CH_2-S-CN

B. PREPARATION OF DOSING SOLUTIONS:

Mixtures of unlabeled and labeled MBT were used throughout this study. Both materials were dissolved together in acetone to obtain the required concentrations. The solutions obtained were dried under Nitrogen gas and were redissolved in polyethylene glycol 400 (PEG 400), at the rates of 3 or 30 mg/5 ml, to be administered to the test animals by gavage (5 ml/kg body weight). The final specific activities were 2.06 to 2.61 mCi/mmol (LD solution) and 0.174 mCi/mmol (HD solution)

II. TEST ANIMALS:

Thirty nine male and 39 female Sprague Dawley rats, the males weighing from 171 to 318 grams and the females from 176 to 228 grams, served as the test species. The animals were given standard laboratory rat diet and water ad libitum. They were kept on a 12:12 hr light/dark cycle, at a target temperature of 21± 2° C.

III. METHODS:

A. STUDY DESIGN:

The study was designed to determine the absorption, distribution, excretion, and metabolic profile (not metabolites identification) of ^{14}C-MBT when administered by oral gavage to rats. The test groups used in the study were as follows:

Group(s)*	Dose Level (mg/kg/day)	Dosing Route	No. Animals M	No. Animals F	Formulation
Ia	3	single oral	9	9	^{14}C labeled
Ib	30	single oral	5	5	^{14}C labeled
IIa	3	single oral	3	3	^{14}C labeled
IIb	30	single oral	3	3	^{14}C labeled
IIc	3	repeated oral (7 days)	3	3	^{14}C labeled
III	3	single oral	1	1	^{14}C labeled
IV	3	repeated oral (14 days)	15	15	^{14}C labeled

* Group A (single LD intravenous) of guidelines 85-1 is ommitted from the study because the test material was insoluble in water and/or physiological saline.

I Excretion studies: Ia and Ib correspond to groups B and D in guidelines 85-1.

II Study of radioactivity levels in plasma: These groups are not required by guidelines 85-1.

III Bile cannulation studies: This group is not required by guidelines 85-1.

IV Bioretention and bioaccumulation study: This group corresponds to group C in guidelines 85-1 except that during the 14-day dosing period the rats were given ^{14}C-MBT instead of unlabeled MBT and that they were not given a dose of ^{14}C-MBT on day 15.

B. STUDIES DESCRIPTION:

1. Excretion studies: (Groups Ia and Ib) All animals were individually housed in all glass metabolism cages. Their urine and feces were collected separately at 6, 24, 48, 72 and 96 hours post-dose. Cages washes were collected every 24 hours. $^{14}CO_2$ in the expired air was absorbed serially in toluene and ethanolamine and collected at 6, 24, and 48 hours post-dose. All animals were killed 96 hours post-dose and the bone, brain, fat, heart, skeletal muscle, stomach and contents, blood cells, residual carcass, testes/ovaries, liver, lung, spleen, kidney, intestines and

contents, and plasma were collected. The total radioactivity content of organs/tissues, urine, feces and expired air were determined by combustion and/or liquid scintillation counting.

2. Study of radioactivity levels in plasma: In the single dose studies (groups IIa and IIb) blood samples were obtained from all animals 0.5, 1, 2, 4, 6, 8, 12 (tail vein), and 24 hours (heart) after dosing and the plasma levels of total radioactivity were measured as described above. All animals were sacrificed 24 hours after dosing. In the repeated dosing study (group IIc) blood samples were obtained daily (1 hr post-dose) for measurements of plasma levels of radioactivity. The animals were sacrificed after the last dosing.

3. Bile cannulation studies: (group III) The bile ducts of 1 male and one female rat were cannulated. The bile was collected hourly, for 24 hours, after a single dosing with ^{14}C-MBT (3 mg/kg). The urine and faeces were collected at 6 and 24 hours post-dose. The rats were sacrificed at 24 hour post-dose. Total radioactivity was determined in the bile, urine, faeces, cage wash, carcass, and GI tract as described above.

4. Bioretention and bioaccumulation study: (group IV) Fifteen male and 15 female rats received single daily dosings of ^{14}C-MBT (3 mg/kg) for 1 to 14 days. Three rats of the same sex were killed at each of the following time points: 24 hours after 1, 3, 7, and 14 dosings and 2, 3, 4, 7, 10, and 14 days after 14 dosings. Their urine, feces,cage wash, tissues, and blood were collected as described above for determination of radioactivity.

5. THIN LAYER CHROMATOGRAPHIC (TLC) STUDIES:

i. Urine Studies: Pooled urine collected either 0-24 hours post dose from male and females rats treated singly with ^{14}C-MBT (LD and HD) or 0-24 hours post-doses 1 and 14 from male and females rats treated repeatedly for 14 days with ^{14}C-MBT were analyzed. Samples of 0.5 ml were dryed under nitrogen gas, resuspended in 0.2 ml of methanol, and chromatographed in butanol:water:acetic acid (5:2:1, v/v/v) solvent system. The radioactivity on the TLC plates was measured with a radio-TLC analyser and by autoradiography. A densitometer was used for quantitative analysis of the autoradiograms. The rf for ^{14}C-MBT in this solvent system was around 0.8 (see attached fig. 12 of report).

ii. Feces Studies: Feces studies were done with pooled feces collected from the same rat groups and at the same time periods as the urine studies. The radioactivity in the samples was extracted into methanol (mean extraction efficiency $\pm$ SD= 60.8% $\pm$ 9.7), the extracts were dried under nitrogen, resuspended in methanol, and chromatographed in 100% dichloromethane. The rf for ^{14}C-MBT in this solvent system was around 0.4 (see attached fig. 10 of report).

iii. Tissues Studies: The liver and kidney were used as representative organs. Tissues samples collected from male and/or female rats (Groups IIa+b, group IV at 24 h post-doses 7 and 14, and group IV at 4 and 14 days post-dose 14) were extracted in methanol and processed for chromatography like the fecal samples. The means extraction efficiencies ± SDs were 31.5% ±6.5 for the liver and 45.5% ±6.1 for the kidney.

iiii. Plasma studies: Pooled plasma collected 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-dose from groups Ia and Ib rats, 1 h post-doses 1, 2, 3, 4, 5 and 6 from group IIc rats , 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose 7 from rats of the latter group, and 24 hours post-dose 1, 3, 7, and 14 from group IV rats were analyzed. Samples (0.15-2 ml) were deproteinized with methanol (10 ml) and centrifuged. The supernatants were processed for chromatography like the fecal samples. The mean extraction efficiency ± SD was 20.6% ± 8.0.

IV. QUALITY ASSURANCE:

A signed Statement of Compliance with GLP dated 4-26-89 and a Signed Statement of Quality Assurance dated 3-20-89 were included in the report.

V. RESULTS:

A. EXCRETION OF TOTAL RADIOACTIVITY: (See Table 1)

After a single administration of either 3 or 30 mg/kg of the radioactive test material the excretion of total radioactivity into urine, feces and expired air was rapid and complete. Most of the test compound (94.87-99.95%) administered was excreted from the animals over a 96-hour period. No sex differences were observed.

1. URINARY EXCRETION: The major route of excretion was via the urine. Most of the total urinary excretion occured between 0 and 24 hours following a single dosing with ^{14}C-MBT. LD males and females respectively excreted 59.4% and 59.92% of the administered dose and HD males and females respectively excreted 65.18% and 64.81% of the administered dose. Some additional excretion occured during the next 72 hours. Over the 96 hours period, 63.36 and 62.92 % of the administered dose were respectively recovered in the urine of the LD males and females. For the HD groups these values were respectively 70.90 and 70.46%. After 14 single daily oral administrations of ^{14}C-MBT (3 mg/kg) to 3 male rats, 6.17% of the total dose was recovered in the urine over a 96-hour period. This amount constituted 72% of the total radioactivity recovered in all external excreta. A comparable percentage (74%) was observed when a single low dose of ^{14}C-MBT was administered to male rats.

2. FECAL EXCRETION: Most of the total fecal excretion also occured

between 0 and 24 hours following a single dosing with ^{14}C-MBT. LD males and females respectively excreted 13.15 and 12.33% of the administered dose and HD males and females respectively excreted 19.47 and 17.87% of the administered dose. Some additional excretion occured during the next 72 hours. Over the 96 hours period, 8.93 and 11.61% of the administered dose were respectively recovered in the feces of the LD males and females. For the HD groups these values were respectively 13.63 and 14.42%. After 14 single daily oral administrations of ^{14}C-MBT (3 mg/kg) to 3 male rats, 2.07% of the total dose was recovered in the feces over a 96-hour period. This amount constituted 24% of the total radioactivity recovered in all external excreta. A comparable value (23%) was observed when a single LD of ^{14}C-MBT was administered to male rats.

3. RADIOACTIVITY IN CAGES WASHES: Radioactivity recovered in the cages washes are mainly from the urine. About 3% of the administered doses (LD and HD) was recovered in the washes over the 96 hour post-dose.

4. $^{14}CO_2$ CONTENT OF THE EXPIRED AIR: During the period 0-48 hour more than 10% of the administered dose was excreted as $^{14}CO_2$ in the expired air.

Table 1. Excretion of Total Radioactivity in the Urine, Feces, and Expired CO_2 as Percent (%) of Dose From Rats 1 and 4 Days Following a Single Oral Administration of ^{14}C-Methylene bis (thiocyanate).

	Recovery, as % of Dose			
Time Interval	0-24 hr		0-96 hr	
Dose Level	3 mg/kg (low)	30 mg/kg (high)	3 mg/kg (low)	30 mg/kg (high)
Male				
Urine	59.40	65.18	63.36	70.90
Feces	13.15	8.93	19.47	13.63
Cage wash	1.37	2.63	2.53	3.81
CO_2	7.75	9.94	13.99*	10.96*
Total	81.67	86.68	99.37	99.30
Female				
Urine	59.92	64.81	62.92	70.46
Feces	12.33	11.61	17.87	14.42
Cage wash	2.53	2.37	3.29	3.15
CO_2	9.84	10.98	10.79*	11.92*
Total	84.62	89.77	94.87	99.95

Urine, feces, and cage wash values are cumulative average of 5 male and 5 female rats dosed with the high dose and 9 male and 9 female rats dosed with the low dose. CO_2 values are cumulative average of 5 male and 5 female rats (low or high dose).
* These $^{14}CO_2$ values were obtained in the time interval 0-48 hr. No $^{14}CO_2$ was collected beyond 48 hours.
Data are excerpted from Tables 1-3 of the report.

B. TISSUE DISTRIBUTION OF TOTAL RADIOACTIVITY

Less than 1% of the administered single doses were found in the tissues of groups Ia and Ib rats. The levels recovered ranged from 0.04-0.25 ug equiv.g^{-1} (ml^{-1}) in the LD and from 0.55 to 3.65 ug equiv.g^{-1} (ml^{-1}) in the HD tissues. Tissues with contents $\geq$ 3 equiv.g^{-1} included the kidney, liver, lung, and spleen. Those with contents $\leq$ 1 equiv.g^{-1} (ml^{-1}) included the plasma, brain, fat, muscle, and testis. all other tissues contained between 1 to <3 equiv.g^{-1} (ml^{-1}). No sex differences were apparent (see table 2).

Table 2. Tissue Distribution of Total Radioactivity in Rats 96 Hours After one Oral Dosing With ^{14}C-MBT.

	ug equiv.g^{-1} (ml^{-1}) (% dose recovered)			
	3 mg/kg		30 mg/kg	
Organ/Tissue	Male	Female	Male	Female
GI tract tissues and contents				
stomach	0.16	0.22	2.20 (0.09)	1.83 (0.12)
s.intestine	0.08	0.09	1.33 (0.23)	1.28 (0.23)
Liver	0.16	0.20	2.65 (0.30)	3.10 (0.42)
Carcass	(2.30)	(2.13)	(2.37)	(2.63)
Blood cells	0.08	0.08	2.05	2.47
Plasma	0.04	0.05	0.58	0.55
Bone	0.09	0.09	1.30	1.16
Brain	0.04	0.04	0.59 (0.01)	0.64 (0.02)
Fat	0.06	0.07	0.89	0.84
Heart	0.11	0.14	1.55 (0.02)	2.03 (0.02)
Kidney	0.21	0.25	3.05 (0.08)	3.65 (0.09)
Muscle	0.09	0.08	0.83	1.02
Spleen	0.18	0.20	2.66 (0.04)	3.17 (0.02)
Testis	0.07		0.84 (0.03)	
Ovary		0.16		2.61 (0.01)
Lung	0.14	0.16	2.18 (0.03)	3.18 (0.05)

Values are average of 5 male and 5 female rats. Data excerpted from Tables 4-6 of the report.

The distribution of total radioactivity in tissues of rats treated with 14 single daily LD of ^{14}C-MBT (group IV) are summarized in table 3. Tissues levels at day 1 post-dose were comparable to those observed 96 hours following a single HD. This suggested a certain increase in accumulation with repeated dosings. Although the radioactivity contents of all tissues declined thereafter, levels $\geq$ 0.60 equiv.g^{-1} (ml^{-1}) were still observed in the kidney, lung, RBC, skeletal muscle, heart, and fat 14 days after the dosings had stopped. All the tissues studied had higher radioactivity contents than the plasma (0.04-0.06 equiv.g^{-1} (ml^{-1}).

Table 3. Distribution of Total Radioactivity in Rats following 14 single daily oral administration of ^{14}C-MBT (3 mg/kg)

Organ/Tissue	ug equiv.g^{-1} (ml^{-1}) Time of Sacrifice (Days Post Dose 14)				
	1a	4a	7a	10b	14b
GI tract tissues and contents					
stomach				0.41	0.45
s.intestine				0.19	0.18
Liver	2.04	0.98	0.70	0.44	0.38
Carcass	0.29	0.27	0.17	0.23	0.14
Blood cells				0.70	0.69
Plasma	0.58	0.23	0.14	0.04	0.06
Bone				0.37	0.22
Brain				0.29	0.30
Fat	1.34	1.18	0.67	1.17	0.68
Heart				0.82	0.89
Kidney	2.59	1.45	1.14	0.72	0.79
Muscle				0.89	0.66
Spleen				0.54	0.55
Testis/Ovaries				0.38	0.48
Lung				0.68	0.51

a Values are average of 6 male and 6 female rats.
b Values are average of 3 male and 3 female rats.
Data excerpted from Tables 12-14 of the report.

C. TOTAL RADIOACTIVITY LEVELS IN PLASMA

Plasma levels of total radioactivity was studied (0.5-24 hours post dose) in both the single LD and HD studies and in the 7-day repeated dosing study with the LD. It was also studied 1 hour post-dose, daily, during the latter study (see table 4). Peak plasma levels occured between 1 and 2 hours post-dose in all cases. Mean peak plasma concentrations were 1.048, 14.15, and 1.908 ug equiv.ml^{-1} respectively for the single LD, single HD, and the 7-day repeated dosing groups. Plasma clearance was rapid. Only 15-16% of the peak levels remained 24 hours after either low or high single dose. A slightly higher level (26% of the peak plasma level) remained 24 hours after the 7th dosing. No sex differences were noted.

Table 4. Plasma levels of total radioactivity following single or repeated oral administrations of ^{14}C-MBT to rats.

	ug equiv.ml^{-} and (% of administered dose)		
Time (hr post dose)	Single LD	Single HD	Repeated LD*
1	1.048 (100)	13.51 (95)	1.790 (94)
2	1.042 (99)	14.15 (100)	1.908 (100)
4	0.822 (78)	12.06 (85)	1.717 (89)
12	0.366 (35)	7.72 (55)	0.884 (46)
24	0.165 (16)	2.19 (15)	0.492 (26)

Values are average of 3 male and 3 female rats.
* times are in hours following the 7th single daily dose.
Data excerpted from tables 8-10 of the report.

D. TOTAL RADIOACTIVITY LEVELS IN BILE: One male and one female rat were given a single LD of ^{14}C-MBT and their bile was collected during the next 24 hour period. The results indicated that less than 3% of the administered dose was excreted in the bile over the whole period. According to the report, this low level suggested that "radiolabeled components excreted via feces may represent unabsorbed material". This conclusion is deemed tentative because of the small number of experimental rats used.

E. THIN LAYER CHROMATOGRAPHIC STUDIES:

1. Radiolabeled Components in Urine: No unchanged ^{14}C-MBT was recovered in any of the urine extracts. The labeled test material appeared to be completely metabolized. A number of radiolabeled components were observed in all the extracts, but none cochromatographed with ^{14}C-MBT. Two of the components (rf 0.55-0.65) were consistently found in every sample and accounted for $\leq$ 53% of the total radioactivity (see attached table 17 and representative figs.13 and 16 of report). The study did not however characterize any of the unidentified radiolabeled components observed.

2. Radiolabeled Components in Feces: None of the fecal extracts from males and female rats treated with the LD, either singly or repeatedly, showed any radiolabeled component with an rf similar to that of ^{14}C-MBT (see attached fig. 22 of report). On the other hand, fecal extracts from male and female rats treated with a HD showed 1 radiolabeled component which co-chromatographed with ^{14}C-MBT (see attached fig. 23 of report). No characterization of any of the unidentified radiolabeled components were conducted.

3. Radiolabeled Components in Plasma, Kidney and liver: None of the observed radiolabelled components were reported to co-chromatograph with ^{14}C-MBT. It should be noted however that the extraction efficiencies for the plasma (20%), kidney (45%), and liver (31%) were low. Consequently all of the break down metabolic products of MBT were not probably accounted for on the TLC plates.

VI. REPORTED CONCLUSIONS/DISCUSSION:

Single doses (3 and 30 mg/kg) of ^{14}C-MBT administered by the oral route to rats appeared to be rapidly absorbed, extensively metabolized, and rapidly excreted. Plasma levels peaked between 1 and 2 hours post-dose to rapidly decline to ≤ 16% in 24 hours. Most of the test material (94.87 - 99.95%) were eliminated in the urine, feces, and expired air in 96 hours. Urinary excretion was the most important route of excretion, accounting for 59.4-65.18% of the administered doses at 24 h post dose and for 62.92-70.90% of the administered doses at 96 h post dose. Fecal excretion accounted for ≤ 20% of the administered doses at 96 h post dose. Excretion by $^{14}CO_2$ accounted for >10% of the administered doses at 48 h post-dose. In the 14-day repeated dosing study, a similar urine/feces radioactivity ratio was observed 14 days after the last dose.

Very small amounts of total radioactivity (<1% of the administered dose) were recovered in the tissues of male and female rats killed either 96 hours after a single oral dose of ^{14}C-MBT (LD or HD) or 14 days after the last of the 14 daily dosings with the LD. Radioactivity was rather uniformly distributed. This widespread distribution, the rather long apparent half life of elimination of radioactivity from the tissues, and the significant recovery of $^{14}CO2$ in the expired air all suggested that the ^{14}C-labelled components may be entered in the endogenous metabolic pathways.

The low level of radioactivity recovered in the bile (<3% of administered dose) suggested that "the radiolabeled components excreted via feces may represent unabsorbed material".

The following are direct quotes from the report regarding the metabolic profile of ^{14}C-MBT: "Chromatographic analysis of radiolabeled components in urine showed apparently complete metabolism of ^{14}C-MBT, with a substantial number of radiolabeled metabolites. At the high dose level, significant levels of a component which co-chromatographed with parent compound was observed in fecal extract, although no such component was observed in fecal extracts from rats after single or multiple administration at the low dose level. No components of similar chromatographic character to parent material were observed in the extracts of tissues and plasma analysed by TLC". "Organic solvent extraction of radiolabeled components in feces, tissues, and plasma was difficult and it is possible that some of the unextractable radioactive material are strongly associated with components in

radioactive material are strongly associated with components in tissues and plasma".

VII. TB Conclusions:

The data submitted was adequate to support the report's conclusions. The toxicological data provided on the absorption, distribution, and excretion of total radioactivity following single or repeated oral dosings with ^{14}C-MBT is acceptable. The data provided on the identification of the metabolites of MBT in the urine, feces, expired air, and tissues is incomplete. Guidelines 85-1 requires a characterization of those metabolites. In this study, the only characterizations done were those of $^{14}CO_2$ in the expired air and of the unchanged MBT in the fecal extracts of group Ib rats. An identification of the metabolites present in the urine, feces, and tissues is required. It is suggested that the registrant use a better extraction system for the feces, plasma, kidney, and liver in view of the poor extraction efficiencies realized with the system (100% dichloromethane) used in this study.

In addition the registrant should provide information on the purity and composition of the unlabeled test material used in this report as this information was not provided in the report.

Lastly, according to guidelines 85-1, the HD should produce some toxic/pharmacologic signs. The registrant should state which adverse effect was observed which validated the use of 30 mg/kg as the HD in this study.

Until all 3 above requirements are satisfied, this study is considered supplementary (85-1).

PAGES 15 THROUGH 21 HAVE BEEN REMOVED. THOSE PAGES CONSIST OF REGISTRANT-SUBMITTED DATA.