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Provisional Peer-Reviewed Toxicity Values for

2-Mercaptobenzothiazole
(CASRN 149-30-4)

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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database uncertainty factor
U.S.	United States of America
WBC	white blood cell

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 2-MERCAPTOBENZOTHIAZOLE (CASRN 149-30-4)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (http://www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVs

Questions regarding the content of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

2-Mercaptobenzothiazole (MBT), CASRN 149-30-4, and its sodium (sodium MBT) and zinc (zinc MBT) salts are primarily used in vulcanization processes as cure-rate accelerators for both natural and synthetic rubber products. These compounds are also used as metal chelators, corrosion inhibitors, and in ore flotation and veterinary drugs (RAPA Panel, 2003). The sodium and zinc salts of MBT are registered fungicides, microbiocides, and bacteriostats; MBT itself was registered as a pesticide active ingredient in 1956 (U.S. EPA, 1994). However, all pesticide products containing MBT and zinc MBT have since been discontinued and only one product (Vancide 51) containing sodium MBT is still registered for use in the United States (Kegley et al., 2014).

MBT is a solid that will exist partially as an anion in aqueous environments, based upon its pKa of 6.93. As anions do not volatilize, volatilization from moist surfaces is not expected. Volatilization from dry surfaces is also unlikely due to MBT's low vapor pressure. The capacity of MBT to leach to groundwater or undergo runoff after a rain event would depend upon local conditions, as displayed by the range of measured soil adsorption coefficients. In addition, the MBT anion may complex with metal ions in the environment to form a less water-soluble metal-anion complex (HSDB, 2010).

The sodium salt, sodium MBT, readily dissociates in water, as evidenced by its high water solubility, to yield an MBT anion and sodium cation. This high solubility indicates that sodium MBT would likely leach to groundwater or undergo runoff after a rain event. However, as with the parent compound MBT, the MBT anion portion may complex with metal ions in the environment to form a less water-soluble metal-anion complex. Conversely, zinc MBT, which has much lower water solubility, is not expected to readily dissociate in water, but rather remain associated as a metal-anion complex. In addition, zinc MBT's moderate water solubility indicates that its propensity to leach to groundwater would be considerably less than that of the sodium salt. Because both compounds are salts, volatilization is not expected to be an important fate process. The empirical formulas for MBT, sodium MBT, and zinc MBT are $C_7H_5NS_2$, $C_7H_4NNaS_2$, and $(C_7H_4NS_2)_2Zn$, respectively (see Figures 1−3). A table of physicochemical properties is provided below (see Table 1).

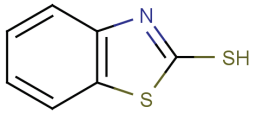

Figure 1. 2-Mercaptobenzothiazole Structure

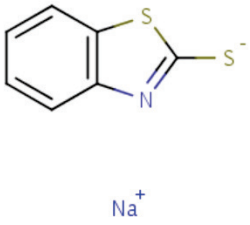

Figure 2. Sodium 2-Mercaptobenzothiazole Structure

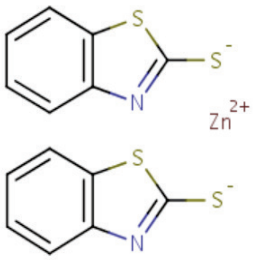

Figure 3. Zinc 2-Mercaptobenzothiazole Structure

Table 1. Physicochemical Properties of MBT, Sodium MBT, and Zinc MBT

Property (unit)	MBT (CASRN 149-30-4)	Sodium MBT (CASRN 2492-26-4)	Zinc MBT (CASRN 155-04-4)
Physical state	Solid[a]	Solid (hygroscopic and prone to oxidation)[a]	Solid[a]
Boiling point (°C)	Decomposes >260[a]	ND	Decomposes >362[a]
Melting point (°C)	181[a]	>300[b]	337[a]
Density (g/cm^3)	1.42[a]	ND	1.7[a]
Vapor pressure (mm Hg at 25°C)	$<2.2 \times 10^{-6}$ [a]	ND	ND
pH (unitless)	ND	10 (1% aqueous solution) >11.5 (50% aqueous solution)[c]	5.55 (1% aqueous suspension)[c]
pKa (unitless)	6.93[b]	ND	ND
Solubility in water (mg/L at 25°C)	118 (at pH 7)[a]	>500,000[a]	90.9 (at 20°C)[a]
Octanol-water partition coefficient (log K_{ow})	2.41[a]	−0.46[a]	ND
Henry's law constant (atm-m^3/mol at 25°C)	4.1×10^{-9} [d]	ND	ND
Soil adsorption coefficient K_{oc} (mL/g)	677−3,560 (measured values in various soils)[a]	ND	ND
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	40.6×10^{-12} [d]	ND	ND
Atmospheric half-life (hr)	9.5[d]	ND	ND
Molecular weight (g/mol)	167.24	189.23	397.7

[a]RAPA Panel (2003).
[b]U.S. EPA (2010).
[c]U.S. EPA (1994).
[d]U.S. EPA (2012c).

MBT = 2-mercaptobenzothiazole; ND = no data.

A summary of available toxicity values for MBT and its sodium and zinc salts from U.S. EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for MBT, Sodium MBT, and Zinc MBT (CASRNs 149-30-4, 2492-26-4, and 155-04-4)

Source (parameter)[a,b]	Value (applicability)	Notes	Reference
Noncancer			
IRIS	NV	NA	U.S. EPA (2016)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
ATSDR	NV	NA	ATSDR (2016)
IPCS	NV	NA	IPCS (2016); WHO (2016)
Cal/EPA	NV	NA	Cal/EPA (2014); Cal/EPA (2016a); Cal/EPA (2016b)
OSHA	NV	NA	OSHA (2006); OSHA (2011)
NIOSH	NV	NA	NIOSH (2015)
ACGIH	NV	NA	ACGIH (2015)
AIHA (WEEL)	5 mg/m^3 (MBT)	8-hr TWA. Based on NOEL of 188 mg/kg in gavage study, converted to inhalation exposure of 1,300 mg/m^3; TWA of 5 selected to protect against small potential for carcinogenic activity.	AIHA (2013)
Cancer			
IRIS	NV	NA	U.S. EPA (2016)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
NTP	NV	NA	NTP (2014)
IARC	NV	NA	IARC (2015)
Cal/EPA	NV	NA	Cal/EPA (2011); Cal/EPA (2016a); Cal/EPA (2016b)
ACGIH	NV	NA	ACGIH (2015)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; AIHA = American Industrial Hygiene Association; ATSDR = Agency for Toxic Substances and Disease Registry; Cal/EPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Informational System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration.
[b]Parameters: WEEL = workplace environmental exposure level.

MBT = 2-mercaptobenzothiazole; NA = not applicable; NOEL = no-observed-effect level; NV = not available; TWA = time-weighted average.

Non-date-limited literature searches were conducted in February 2016 for studies relevant to the derivation of provisional toxicity values for MBT (CASRN 149-30-4). Searches also included names and CASRNs for the sodium and zinc salts of MBT (CASRNs 2492-26-4 and 155-04-4, respectively). The searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, ToxLine (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related values: ACGIH, ATSDR, Cal/EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA Office of Water (OW), U.S. EPA TSCATS2/TSCATS8e, NIOSH, NTP, and OSHA.

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide overviews of the relevant noncancer and cancer databases (respectively) for MBT. The tables include all potentially relevant repeat-dose, short-term-, subchronic-, and chronic-duration studies, as well as reproductive and developmental toxicity studies. Principal studies are identified in bold. The phrase "statistical significance," used throughout the document, indicates a p-value of < 0.05 unless otherwise noted.

Table 3A. Summary of Potentially Relevant Noncancer Data for MBT (CASRN 149-30-4)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Human								
1. Oral (mg/kg-d)								
ND								
2. Inhalation (mg/m^3)								
ND								
Animal								
1. Oral (mg/kg-d)[b]								
Short-term	5 M/5 F, S-D rat, range-finding study, diet, 4 wk	0, 5,000, 10,000, 15,000, 20,000, 25,000 ppm ADD (M): 0, 425, 839, 1,232, 1,696, 2,143; ADD (F): 0, 432, 874, 1,320, 1,703, 2,058	Reduced body weight and increased relative and absolute liver weight in males and females were accompanied by reduced food intake; these changes may have resulted from poor palatability of the diet, so effect levels were not determined	ND	NA	ND	Monsanto (1989b)	NPR
Short-term	5 M/5 F, F344/N rat, gavage, 5 d/wk, 16 d	0, 156, 313, 625, 1,250, 2,500 mg/kg ADD: 0, 117, 235, 469, 937.5, 1,875	Reduced body-weight gain in males and females (1,875 mg/kg-d)	ND	NA	ND	NTP (1988)	PR
Short-term	5 M/5 F, B6C3F$_1$ mouse, gavage, 5 d/wk, 16 d	0, 188, 375, 750, 1,500, 3,000 mg/kg ADD: 0, 141, 281, 563, 1,125, 2,250	Decreased survival, lethargy, prostration in females	ND	NA	FEL: 1,125	NTP (1988)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for MBT (CASRN 149-30-4)								
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[b]**	**Critical Effects**	**NOAEL[b]**	**BMDL/ BMCL[b]**	**LOAEL[b]**	**Reference (comments)**	**Notes[c]**
Subchronic	12 M/12 F, S-D rat, neurotoxicity study, diet, 13 wk	0, 5,000, 15,000, 25,000 ppm ADD (M): 0, 323.5, 991.1, 1,639.1; ADD (F): 0, 384.4, 1,129.5, 1,920.4	Terminal body weight was reduced by >11% in high-dose females; however, reduced food intake was also seen. FOB, motor activity, and nervous system histopathology results were not affected by exposure. Small (3−5%) decreases in brain weight, length, and width were seen in males at 1,639.1 mg/kg-d, but not in high-dose females. As the body-weight change may have resulted from poor palatability of the diet, effect levels were not determined	ND	NA	ND	Bio-Research Laboratories LTD (1990)	NPR
Subchronic	**10 M/10 F, F344/N rat, gavage, 5 d/wk, 13 wk**	**0, 188, 375, 750, 1,500 mg/kg** **ADD: 0, 134, 268, 536, 1,071**	**Increased absolute and relative liver weight (≥10%) in females and males**	**ND**	**14.8 (relative liver weight in females)**	**134**	**NTP (1988)**	**PR, PS**
Subchronic	10 M/10 F, $B6C3F_1$ mouse, gavage, 5 d/wk, 13 wk	0, 94, 188, 375, 750, 1,500 mg/kg ADD: 0, 67, 134, 268, 536, 1,071	Increased absolute and relative liver weight (≥10%) in females	ND	NA	67 FEL: 536	NTP (1988)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for MBT (CASRN 149-30-4)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Chronic	50 M/50 F, F344/N rat, gavage, 5 d/wk, 103 wk	M: 0, 375, 750 mg/kg F: 0, 188, 375 mg/kg ADD (M): 0, 268, 536; ADD (F): 0, 134, 268	Decreased survival, markedly increased tumor incidences, and forestomach lesions were seen in male rats at >267.86 mg/kg-d; in females, forestomach lesions and increased tumor incidences were seen at 267.86 mg/kg-d. Because 267.86 mg/kg-d was associated with reduced survival in males and tumors in males and females, it cannot be identified as a LOAEL; likewise, the lowest dose in females was associated with a high tumor incidence and thus cannot be identified as a NOAEL	ND	NA	ND	NTP (1988) (Not a comprehensive evaluation of endpoints; hematology, clinical chemistry, and organ weights were not evaluated)	PR
Chronic	50 M/50 F, B6C3F$_1$ mouse, gavage, 5 d/wk, 103 wk	0, 375, 750 mg/kg ADD: 0, 268, 536	Decreased survival of male and female mice at 535.7 mg/kg-d, beginning early in the study and in the absence of tumors. An increased incidence of hepatocellular adenomas was seen in female mice at 267.86 mg/kg-d, precluding the identification of this dose as a NOAEL despite the lack of non-neoplastic effects	ND	NA	FEL: 536	NTP (1988) (Not a comprehensive evaluation of endpoints; hematology, clinical chemistry, and organ weights were not evaluated)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for MBT (CASRN 149-30-4)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Chronic	30 M/30 F (treated), 60 M/60 F (control), Slc:ddY mouse, diet, 20 mo	0, 30, 120, 480, 1,920 ppm ADD (M): 0, 3.60, 14.69, 57.90, 289.40; ADD (F): 0, 3.61, 13.52, 58.82, 247.98	Increased kidney interstitial cell infiltration at ≥57.90 mg/kg-d in males. No effect levels identified due to numerous study limitations	ND	NA	ND	Ogawa et al. (1989); Garcia (2004) (Limited information available, published in Japanese with limited information in a secondary report)	NPR
Reproductive	28 M/28 F, S-D rat, diet, at least 70 d prior to mating, through two generations	0, 2,500, 8,750, 15,000 ppm ADD (M): 0, 172.1, 602.3, 1,033; ADD (F): 0, 199.7, 699.0, 1,198	Increased incidence of basophilic tubules of the renal cortex in F0 males and increased relative liver weight (12%) in F1 male parents	ND	NA	172.1	Springborn Laboratories (1990b)	NPR
Developmental	0 M/6 F, S-D rat, gavage, GDs 6−15	0, 300, 600, 1,000, 1,500, 2,200 ADD: 0, 300, 600, 1,000, 1,500, 2,200	Decreased maternal survival	ND	NA	FEL: 2,200	Springborn Laboratories (1989c)	NPR
Developmental	0 M/5 F, NZW rabbit, gavage, GDs 6−18	0, 150, 300, 600, 1,000, 1,500 ADD: 0, 150, 300, 600, 1,000, 1,500	Decreased maternal survival (≥600 mg/kg-d). Decreased maternal body weight (≥600 mg/kg-d) and fetal body weight (≥150 mg/kg-d). Increased clinical signs of toxicity in dams (emaciation at ≥600 mg/kg-d)	Develop-mental: ND	NA	Maternal FEL: 600 Develop-mental: 150	Springborn Laboratories (1989b)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for MBT (CASRN 149-30-4)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Developmental	0 M/26 F, S-D rat, gavage, GDs 6−15	0, 300, 1,200, 1,800 ADD: 0, 300, 1,200, 1,800	Clinical signs of toxicity and decreased activity in dams; increased postimplantation loss	Maternal: 1,200 Develop-mental: NA	NA	Maternal: 1,800 Develop-mental: 300	Springborn Laboratories (1989e)	NPR
Developmental	0 M/20 F, NZW rabbit, gavage, GDs 6−18	0, 50, 150, 300 ADD: 0, 50, 150, 300	No maternal or developmental effects	Maternal and develop-mental: 300	NA	ND	Springborn Laboratories (1989d)	NPR
2. Inhalation (mg/m^3)[a]								
ND								

[a]Category (treatment/exposure duration: unless otherwise noted): Short-term = repeated exposure for >24 hours ≤30 days (U.S. EPA, 2002) long-term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (more than 30 days up to approximately 90 days in typically used laboratory animal species) (U.S. EPA, 2002); chronic = repeated exposure for >10% lifespan for humans (more than approximately 90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b]Dosimetry: Values are presented as ADDs (mg/kg-day) for oral noncancer effects. In contrast to other repeated exposure studies, values from animal gestational exposure studies are not adjusted for exposure duration in calculation of the ADD.
[c]Notes: PS = principal study; PR = peer reviewed; NPR = not peer reviewed.

ADD = adjusted daily dose; BMCL = benchmark concentration lower confidence limit; BMDL = benchmark dose lower confidence limit; F = female(s); FEL = frank effect level; FOB = functional observational battery; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; M = male(s); MBT = 2-mercaptobenzothiazole; NA = not applicable; ND = no data; NOAEL = no-observed-adverse-effect level; NZW = New Zealand white; S-D = Sprague-Dawley.

Table 3B. Summary of Potentially Relevant Cancer Data for MBT (CASRN 149-30-4)						
Category	**Number of Male/Female, Strain, Species, Study Type, and Duration**	**Dosimetry[a]**	**Critical Effects**	**BMDL/ BMCL[a]**	**Reference (comments)**	**Notes[b]**
Human						
1. Oral (mg/kg-d)						
ND						
2. Inhalation (mg/m^3)[a]						
Carcinogenicity	363 M/0 F, occupational epidemiology, workers exposed at least 6 mo during 1955−1984 (follow-up through 2005)	Exposure categorized as 0, 0.1−1, 1−2.5, 2.5−6, or 6−20 mg/m^3 based on limited monitoring data; individual cumulative exposures estimated as 0, 0.01−21.24, 21.25−63.74, or ≥63.75 mg/m^3-yr	Increased SMRs for colon (SMR = 232, 95% CI = 100−457) and bladder (SMR = 374, 95% CI = 162−737) cancers compared with national mortality rates. Increased SRRs for cancer of the bladder (SRR = 253, 95% CI = 131−441) and multiple myeloma (4 cases, SRR = 465, 95% CI = 127−1,191) compared with national cancer incidence rates. Significant ($p < 0.05$) trends for increasing adjusted RR of colon cancer and multiple myeloma with increasing cumulative MBT exposure	NA	Sorahan and Pope (1993); Sorahan et al. (2000); Sorahan (2009, 2008) (Workers had potential coexposure to other chemicals including *ortho*-toluidine, aniline, and PBN)	PR
Carcinogenicity	1,059 M/0 F, occupational epidemiology, workers exposed ≥1 d between 1955−1977 (follow-up through 1996)	Exposure categorized as 0, >0−0.5, >0.5−2.0, >2.0−5.0, or >5.0−20.0 mg/m^3; cumulative exposure estimated as 0, 0.01−1.9, 2−7.9, or 8−129 mg/m^3-yr	Increased SMR (SMR = 8.9, 95% CI = 4.7−15.2) for bladder cancer in entire MBT-exposed group ($n = 600$). In subgroup with no possible coexposure to PAB ($n = 270$), there were no bladder cancers. In subgroups with likely or potential coexposure to PAB ($n = 89$ and 511, respectively), bladder cancer SMRs were elevated (SMRs = 27.1 and 4.3, respectively). In the subgroup with potential but unknown coexposure to PAB, SMR for bladder cancer increased with cumulative exposure to MBT ($p = 0.04$ for linear trend)	NA	Collins et al. (1999); Strauss et al. (1993) (An unknown proportion of the MBT group had coexposure to PAB, and the highest MBT exposures were during the time when PAB was also used in the plant)	PR

Table 3B. Summary of Potentially Relevant Cancer Data for MBT (CASRN 149-30-4)						
Category	**Number of Male/Female, Strain, Species, Study Type, and Duration**	**Dosimetry[a]**	**Critical Effects**	**BMDL/ BMCL[a]**	**Reference (comments)**	**Notes[b]**
Animal						
1. Oral (mg/kg-d)[a]						
Carcinogenicity	**50 M/50 F, F344/N rat, gavage, 5 d/wk, 103 wk**	**M: 0, 375, 750 mg/kg; F: 0, 188, 375 mg/kg** **HED (M): 0, 64.3, 129; HED (F): 0, 32.2, 64.3**	**Statistically significantly increased incidences of pituitary gland adenomas and adrenal gland pheochromocytomas in females, and statistically significantly increased incidences of mesothelioma, mononuclear cell leukemia, and tumors of the pituitary gland, adrenal gland, pancreas, preputial gland, and subcutaneous tissue in males**	**8.91 (combined tumors in females)**	**NTP (1988)**	**PS, PR**
Carcinogenicity	50 M/50 F, B6C3F$_1$ mouse, gavage, 5 d/wk, 103 wk	0, 375, 750 mg/kg HED: 0, 37.5, 75.0	Statistically significantly increased incidence of hepatocellular adenomas or carcinomas (combined) in low-dose females	NA	NTP (1988)	PR
Carcinogenicity	18 M/18 F C57BL/6 × C3H/Anf, strain "X" and 18 M/18 F C57BL/6 × AKR, strain "Y" mouse, MBT by gavage at 100 mg/kg-d from PNDs 7−28 and diet at 323 ppm thereafter, 18 mo	M: 0, 57.4; F: 57.7 mg/kg-d HED (M): 0, 8.04; HED (F): 8.08	No statistically significant increase in incidence of any tumor type	NA	Innes et al. (1969)	PR

Table 3B. Summary of Potentially Relevant Cancer Data for MBT (CASRN 149-30-4)

Category	Number of Male/Female, Strain, Species, Study Type, and Duration	Dosimetry[a]	Critical Effects	BMDL/ BMCL[a]	Reference (comments)	Notes[b]
Carcinogenicity	18 M/18 F C57BL/6 × C3H/Anf, strain "X" and 18 M/18 F C57BL/6 × AKR, strain "Y" mouse, zinc MBT by gavage at 1,000 mg/kg-d from PNDs 7−28 and diet at 3,385 ppm thereafter, 18 mo	M: 0, 252; F: 253 mg/kg-d as MBT equivalent HED (M): 0, 35.3; HED (F): 35.4	No statistically significant increase in incidence of any tumor type	NA	Innes et al. (1969)	PR
2. Inhalation (mg/m^3)						
ND						

[a]Dosimetry: The units for oral exposures are expressed as human equivalent doses (HEDs) in mg/kg-day. HED = animal dose (mg/kg-day) × $(BW_a \div BW_h)^{1/4}$.
[b]Notes: PS = principal study; PR = peer reviewed.

BMCL = benchmark concentration lower confidence limit; BMDL = benchmark dose lower confidence limit; CI = confidence interval; F = female(s); HED = human equivalent dose; M = male(s); MBT = 2-mercaptobenzothiazole; NA = not applicable; ND = no data; PAB = 4-aminobiphenyl; PBN = phenyl-β-naphthylamine; PND = postnatal day; RR = relative risk; SMR = standardized mortality ratio; SRR = standardized rate ratio.

HUMAN STUDIES

Oral Exposures

No studies have been identified.

Inhalation Exposures

No studies investigating the noncancer effects of exposure to MBT in humans have been identified. Several studies investigated cancer morbidity and mortality, especially bladder cancer, in two cohorts of rubber factory workers with MBT inhalation exposure. Sorahan and colleagues (Sorahan, 2009, 2008; Sorahan et al., 2000; Sorahan and Pope, 1993) reported several analyses of a cohort of 2,160 workers employed at least 6 months between 1955 and 1984 in a chemical production facility in Ruabon, Wales, where vulcanization inhibitors and accelerants and other rubber industry materials were produced. Strauss and colleagues (Collins et al., 1999; Strauss et al., 1993) published two analyses of cancer mortality in 1,059 male workers employed for at least 1 day between 1955 and 1977 at a similar manufacturing facility in Nitro, WV. The studies of these cohorts suffer from several limitations that limit the ability to draw firm conclusions regarding the association between MBT exposure and cancer in these workers. These limitations included: (1) the numbers of workers with likely MBT exposure in both cohorts were small (≤600 workers each), and (2) the numbers of tumors observed were likewise small. Also, the possibility of confounding is high. Both worker cohorts had potential exposure to MBT, its derivatives, and other chemicals, including the known or suspected bladder carcinogens, 4-aminobiphenyl (PAB), and phenyl-β-naphthylamine (PBN). MBT exposure assessments for both cohorts were based on job-exposure matrices, using limited exposure monitoring information, and both included workers who may have had very brief exposure. In addition, data on tobacco use were not available for either cohort. Finally, follow-up studies of the same cohort and studies of the different cohorts did not provide consistent findings, possibly because the numbers of cases and the sizes of the cohorts were too small to yield stable results. The most recent analyses of these cohorts (Sorahan, 2009, 2008; Collins et al., 1999) are discussed here, as they provided the longest follow-up times and addressed the same cancer endpoints as earlier analyses.

Sorahan (2008) and Sorahan (2009) evaluated cancer mortality and incidence in a subcohort of the Wales factory workers consisting of those workers in job categories that had probable exposure to MBT. Sorahan (2009) reported the results of all cancers, while Sorahan (2008) reported the analysis of bladder cancer in particular. The subcohort of 363 workers (from the entire cohort of 2,160) included 37 workers also believed to have had exposure to PBN, 24 workers believed to have had exposure to *o*-toluidine, and 8 workers believed to be exposed to all three compounds (Sorahan et al., 2000). The subcohort of 363 workers studied by Sorahan (2008) and Sorahan (2009) included 6 workers initially considered unexposed to MBT but reclassified as exposed (Sorahan, 2008); earlier studies (Sorahan et al., 2000; Sorahan and Pope, 1993) reported the number of MBT-exposed workers as 357. Some members of the entire cohort were believed to have been exposed to aniline; however, none of the studies indicated whether there were job descriptions with exposure to both MBT and aniline. Exposures to MBT and its derivatives were estimated as described in Sorahan and Pope (1993) and were categorized in this study as zero exposure, very-low exposure (0.1−1 mg/m^3), low exposure (1−2.5 mg/m^3 or <21.25 mg/m^3-years), medium exposure (2.5−6 mg/m^3 or 21.25−63.74 mg/m^3-years), or high exposure (6−20 mg/m^3 or >63.75 mg/m^3-years). Cancer morbidity and mortality rates in the exposed cohort were compared to those observed in a control population of 1,797 unexposed

workers in the same plant, as well as to those rates calculated from the general population of England and Wales. Standardized mortality ratios (SMRs) and standardized ratio rates (SRRs) were calculated using indirect standardization and Poisson regression analysis. Based on national mortality rates, significant excess mortality for cancers of the colon (eight cases, SMR = 232, 95% confidence interval [CI] = 100−457) and bladder (eight cases, SMR = 374, 95% CI = 162−737) were estimated. Based on national cancer incidence rates, significant excess morbidity was estimated for cancer of the bladder (12 cases, SRR = 253, 95% CI = 131−441) and multiple myeloma (four cases, SRR = 465, 95% CI = 127−1,191). Nonsignificant increases in the SRRs for colon and lung cancers were also noted.

Using the internal comparison group (members of the cohort without MBT exposure), significant ($p < 0.05$) trends for increasing adjusted (for age, calendar year, and exposure to other compounds) relative risk (RR) with increasing cumulative MBT exposure were observed for colon cancer and multiple myeloma (Sorahan, 2009), but not for lung cancer (Sorahan, 2009) or bladder cancer (Sorahan, 2008). The adjusted RRs among those with greatest cumulative exposure to MBT were 4.69 (95% CI = 1.38−15.90, three cases) for colon cancer and 20.57 (95% CI = 2.58−164, two cases) for multiple myeloma (Sorahan, 2009).

Both Collins et al. (1999) and Strauss et al. (1993) evaluated mortality in a cohort of workers at the Nitro, WV facility; Collins et al. (1999) followed the cohort through December 1996. A total of 600 out of the 1,059 workers were exposed to MBT. For workers exposed to MBT, a detailed exposure assessment was performed, in which average annual air concentrations were estimated for all jobs by an industrial hygienist using sampling data, employee interviews, and company documents (Strauss et al., 1993). To account for the potential confounding of PAB exposure on bladder cancer mortalities reported in Collins et al. (1999), Strauss et al. (1993) grouped workers exposed to MBT by their job category or time of employment, in an effort to assess potential coexposure to PAB, using the following categories: (1) workers with jobs with exposure to PAB ($n = 89$), (2) workers without jobs with exposure to PAB but including workers with plant-wide jobs with potential PAB exposure ($n = 511$), and (3) workers employed after PAB use was discontinued ($n = 270$, a subset of Group 2). Cumulative MBT exposure was stratified as follows: no exposure, and 0.01−1.9, 2−7.9, and 8−129 mg/m^3-years. SMRs were calculated, using the mortality experience of the white male population of four counties within 20 miles of the facility as the referent rate.

SMRs for the entire cohort were reported; however, this group included workers without MBT exposure (Collins et al., 1999). SMRs for lung, prostate, and bladder cancer were calculated for the 600 MBT-exposed workers. In those exposed to MBT, a significantly increased SMR was observed for bladder cancer (SMR = 8.9, 95% CI = 4.7−15.2), while prostate and lung cancer SMRs were not elevated. In subgroups with likely or possible coexposure to PAB (1 and 2), bladder cancer SMRs were also significantly elevated (SMR = 27.1, 95% CI = 11.7−53.4 and 4.3, 95% CI = 1.4−10.0, respectively). In the group of 270 workers with MBT exposure and no possible exposure to PAB (Group 3), there were no bladder cancers. In Subgroup 2, the SMR for bladder cancer increased with cumulative exposure to MBT ($p = 0.04$ for linear trend). Because an unknown proportion of this group had coexposure to PAB, and the highest MBT exposures were during the time when PAB was also used in the plant, Collins et al. (1999) concluded that the potential confounding made it difficult to assess the risk of bladder cancer attributable to MBT exposure.

MBT is well known to cause contact allergic dermatitis in humans exposed dermally, and the compound is included in the standard patch test allergy panel (Diepgen et al., 2006; Baer et al., 1973). A large number of case reports and case series describing allergic dermatitis in humans exposed to MBT are available, but they are not reviewed here because their usefulness for deriving provisional oral and inhalation toxicity values is limited.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to MBT have been evaluated in short-term-duration studies (Monsanto, 1989b; NTP, 1988), subchronic-duration studies (Bio-Research Laboratories LTD, 1990; NTP, 1988), chronic-duration studies (Garcia, 2004; Ogawa et al., 1989; NTP, 1988; Innes et al., 1969), reproductive toxicity studies (Springborn Laboratories, 1990b), and developmental toxicity studies (Springborn Laboratories, 1989b, c, d, e).

Short-Term-Duration Studies

Monsanto (1989b)

In an unpublished range-finding study (Monsanto, 1989b), groups of Sprague-Dawley (S-D) rats (five/sex/group) were administered MBT (97.6% purity) at target concentrations of 0, 5,000, 10,000, 15,000, 20,000, or 25,000 ppm in the diet, for approximately 4 weeks. Average daily test material intakes estimated by the study authors were 0, 425, 839, 1,232, 1,696, or 2,143 mg/kg-day, respectively, in males and 0, 432, 874, 1,320, 1,703, or 2,058 mg/kg-day, respectively, in females. All animals were observed twice daily for mortality and moribundity. Detailed observations for clinical signs of toxicity, and recording of body weights and food consumption, were performed weekly. All animals were sacrificed at the end of the exposure period and examined for grossly visible external and internal abnormalities. The liver was collected from each animal and weighed; no other organs were weighed. Statistical analyses were conducted, including Dunnett's test and Bartlett's test.

No unscheduled deaths were reported by Monsanto (1989b). There were no test substance-related clinical signs during the exposure period. Effects of exposure on body weight were first noted during Week 1 in males and females. Cumulative body-weight gains over the 4-week period were statistically significantly reduced in males at ≥1,232 mg/kg-day (increasing with dose from 17−21% lower than controls) and in females at 1,703 mg/kg-day (26−41%). The decreases in cumulative body-weight gain were correlated with statistically significant reductions in food consumption in males (11−12%) and females (11%) at the same doses. Terminal body weights were biologically significantly decreased (10−12% lower than controls) in males exposed to ≥1,696 mg/kg-day, but only in females exposed to 1,703 mg/kg-day (and not 2,058 mg/kg-day). No effects of treatment on food efficiency (weight gain as a function of food intake) were observed in males or females. Relative liver weights were biologically significantly increased in males (17−28% higher than controls) and in females (15−30%) at all doses. Absolute liver weights were biologically significantly increased in males at all doses except 1,232 mg/kg-day (11−15%) and in females at ≥874 mg/kg-day (14−19%). The greater increases in relative liver weight compared to absolute liver weight may have been due, in part, to reduced body weight. No test substance-related macroscopic abnormalities were seen at necropsy; microscopic examinations were not performed. Because the reduced body weight and increased liver weight were accompanied by reduced intake of food containing the test compound, these

changes may have resulted from poor palatability of the test material. Consequently, adverse effect levels were not determined for this study.

NTP (1988)

NTP (1988) conducted two range-finding studies in groups of F344/N rats and B6C3F$_1$ mice (five/sex/group) administered MBT (96−97% purity), 5 days/week, for 16 days (12 doses over 16 days). In the first study, groups of rats and mice were administered 0, 156, 313, 625, 1,250, or 2,500 mg/kg of MBT in corn oil by gavage. Adjusted daily doses (ADDs)[1] are 0, 117, 235, 469, 937.5, or 1,875 mg/kg-day, respectively. In the second study, B6C3F$_1$ mice were administered 0, 188, 375, 750, 1,500, or 3,000 mg/kg in corn oil by gavage. ADDs are 0, 141, 281, 563, 1,125, or 2,250 mg/kg-day, respectively. All animals in both studies were observed twice daily and weighed on Days 1, 8, and 15. All animals were sacrificed at the end of exposure. Hematology, clinical chemistry, and organ weights were not measured. Histology was performed on all vehicle control and high-dose (1,875 mg/kg-day) male rats, one rat exposed to 235 mg/kg-day, and one high-dose female rat (1,875 mg/kg-day). Histology was not performed on any mice in either study.

In rats, no chemical-related deaths occurred. Mean body-weight gains were lower (6−7 g, 8−14%) in high-dose rats (1,875 mg/kg-day) of both sexes compared to controls. No chemical-related lesions were reported in rats.

Results for mice from the first study were not reported due to "an excessive number of gavage accidents." In the second study, 4/5 male and 5/5 female high-dose mice (2,250 mg/kg-day) and 4/5 female mice administered 1,125 mg/kg-day died; lethargy and prostration were reported after the first gavage dosing of these mice (>1,125 mg/kg-day). Terminal body weight and body-weight gains were not different between control and treated mice. No chemical-related lesions were reported in the mice. Due to the changes in body-weight gain and survival, NTP (1988) chose doses of 0, 94 (mice only), 188, 375, 750, or 1,500 mg/kg for the subchronic-duration study. Based on these data in mice, 1,125 mg/kg-day is a frank effect level (FEL) for mortality (4/5 female mice died).

Subchronic-Duration Studies

Bio-Research Laboratories LTD (1990)

In an unpublished neurotoxicity study, groups of S-D rats (12/sex/group) were exposed to MBT (purity not available[2]) at dietary concentrations of 0, 5,000, 15,000, or 25,000 ppm for 13 weeks (Bio-Research Laboratories LTD, 1990). Average test material intakes estimated by the study authors were 0, 323.5, 991.1, or 1,639.1 mg/kg-day, respectively, in males and 0, 384.4, 1,129.5, or 1,920.4 mg/kg-day, respectively, in females. All animals were examined at least twice daily for mortality and clinical signs of toxicity. Results of detailed clinical observations and body weights were recorded weekly. Food consumption was measured once weekly for the control, mid-dose, and high-dose groups and twice weekly for the low-dose group. All animals were subjected to a functional observational battery (FOB) prior to exposure; at 1, 6, and 24 hours after initiation of the test diets; and again on study Days 7, 14, 35, 64, and 91. Qualitative FOB parameters evaluated included the following: observations in the chamber

[1]ADDs were calculated by multiplying by 12 doses and dividing by 16 days (e.g., 156 mg/kg × (12 ÷ 16) = 117 mg/kg-day).
[2]According to the table of contents, pp. C341–C617 contained purity information; however, these pages were missing from the available copy of the document.

(body position, locomotor activity, bizarre behavior [static], tremors, twitches, convulsions, piloerection, respiratory rate/pattern, and defecation), handling observations (vocalization, pupil size, lacrimation, salivation, urinary staining, diarrhea, body tone, abdominal tone, pinna reflex, corneal reflex, extensor thrust, tail/toe pinch, and auricular startle), observations in an arena (defecation, gait, bizarre behavior [movement], and limb rotation), observations at the edge of an arena (positional passivity), surface observations (olfactory response, visual placing, and air righting reflex), and observations under the cage (urination). Quantitative FOB parameters included forelimb and hindlimb grip strength and hindlimb splay. All animals were examined for effects on motor activity prior to initiation, and on study Days 29, 63, and 90.

At scheduled necropsy, six animals/sex/group from the control and high-dose groups were anaesthetized and perfused for neuropathological evaluations; brain weight, length, and maximum width were recorded. The following tissues were examined microscopically in the selected animals: brain (forebrain, center of the cerebrum, midbrain, cerebellum and pons, and medulla oblongata), spinal cord (cervical and lumbar swelling), skeletal muscle (e.g., gastrocnemius), and peripheral nervous system (sciatic nerve, lumbar dorsal root ganglion and fibers, lumbar ventral root fibers, cervical dorsal root ganglion and fibers, cervical ventral root fibers, sural nerve, tibial nerve, and Gasserian ganglion). No other organs were weighed or examined microscopically. The remaining six animals/sex/group were examined for gross pathological changes. Statistical analysis of continuous endpoints employed analysis of variance (ANOVA) followed by Dunnett's test (when variances were not significantly different by Bartlett's test) or Kruskal-Wallis test followed by Dunn's test (when variances differed). Repeated measures analysis was used for motor activity counts, and quantal data were analyzed using Fisher's exact test (with alpha adjusted for multiple comparisons).

No mortalities occurred during the study, and there were no treatment-related clinical signs (Bio-Research Laboratories LTD, 1990). Mean body weights were statistically significantly decreased in males at 991.1 mg/kg-day on Days 1 and 7; in high-dose males and mid- and high-dose females, mean body weights were statistically significantly decreased throughout most of the study. Terminal body weights were 9% lower than controls in high-dose males, 7% lower in mid-dose females, and 11% lower than controls in high-dose females. Mean food consumption was statistically significantly decreased in males during Weeks 1 (mid and high doses) and 13 (high dose only); in females, statistically significant decreases in food consumption were noted at the mid dose during Weeks 1 and 5 and at the high dose during Weeks 1, 2, and 5−9). The food intake differences from control were as high as 20% on isolated occasions; in high-dose females, the decrease was between 8 and 13% during Weeks 5−9. No test substance-related effects on FOB or motor activity parameters were observed. Statistically significant decreases in brain weight (5% lower than controls), length (3%), and width (3%) were observed in high-dose males (lower dose groups were not examined). These decreases in brain measures corresponded to lower terminal body weights; there were no differences in relative brain weight between control and high-dose males. In females, no statistically significant differences in brain weight, length, or width occurred at any dose. There were no exposure-related effects on nervous system tissue histopathology. As with the 4-week dietary study in rats conducted by Monsanto (1989b), it is plausible that the body-weight decrements seen in this study were related to the decreases in food consumption and poor palatability. Further, the biological significance of small (3−5%) differences in brain morphometry is uncertain. Thus, effect levels were not determined for this study.

NTP (1988)

NTP (1988) conducted a 13-week study in rats and mice to evaluate the effects of repeated administration of MBT (96−97% purity) and to determine the doses to be used in the 2-year studies. Groups of 10 male and 10 female F344/N rats and $B6C3F_1$ mice were administered 0, 94 (mice only), 188, 375, 750, or 1,500 mg/kg of MBT in corn oil by gavage, 5 days/week, for 13 weeks. ADDs (from 5−7 days) are 0, 67 (mice only), 134, 268, 536, or 1,071 mg/kg-day, respectively. All animals were observed for mortality or moribundity twice daily and weighed weekly. Hematology and clinical chemistry were not evaluated. At sacrifice at the end of exposure, gross necropsies were performed on all animals excluding those that had been cannibalized or had undergone extreme autolysis. Liver weights were recorded, but no other organ weights were obtained. Histopathology examinations were performed on some, but not all, animals from all dose groups. The following tissues were examined: adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), gall bladder (mice only), heart, kidneys, liver, lungs and bronchi, mammary glands, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate and testes or ovaries and uterus, salivary glands, small intestine, spleen, spinal cord, sternebrae or femur or vertebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Any tissues with gross lesions or masses were also examined.

NTP (1988) reported that no compound-related deaths occurred among rats. Gavage error deaths included one male in the 268-mg/kg-day group and two in the 1,071-mg/kg-day group, and one female in the 134-mg/kg-day group and two in the 536-mg/kg-day group. Clinical signs of toxicity consisting of irritable behavior that increased in severity with increasing dose were observed (incidences not reported). The study authors suggested that this behavior reflected resistance to gavage; however, as the clinical signs in rats were more pronounced at higher doses, and clinical signs suggestive of neurotoxicity were seen in the 13-week study in mice (described below), a compound-related neurotoxic response cannot be ruled out. Necropsy body weights were slightly lower (3−9%) than controls in males at doses ≥268 mg/kg-day and in females at all dose groups, but the difference was <10% in all groups (see Table B-1). Body-weight gain also decreased with increasing dose (7−17%) compared to controls. Relative liver weights were statistically significantly increased (14−36% relative to controls) in both male and female rats of all treated groups (see Table B-1). Absolute liver-weight increases (>10%) occurred in all dose groups in females (18−27% higher than controls) and males (15−38% higher than controls). The study authors reported that no treatment-related gross or microscopic lesions were seen (data not shown). Based on >10% increased relative and absolute liver weights in male and female rats, a lowest-observed-adverse-effect level (LOAEL) of 134 mg/kg-day (the lowest dose tested) is identified for this study. A no-observed-adverse-effect level (NOAEL) is not identified.

In mice, 5/10 males and 7/10 females from the 1,071-mg/kg-day group, as well as 2/10 females from the 536-mg/kg-day group, died prematurely (see Table B-2) (NTP, 1988). Two of the deaths, apparently in the high-dose group[3], were due to gavage error. The cause(s) of the remaining deaths was not reported, but is assumed to be related to treatment, especially given the observation of clinical signs including clonic seizures, lacrimation, and salivation in these

[3]The text in the results section of the NTP (1988) 13-week study in mice only mentioned the high-dose deaths, and reported that two of the deaths were due to gavage error. The summary table in that section (see Table 22) also shows deaths of two females in the 750-mg/kg (535.7 mg/kg-day adjusted dose) group.

two dose groups. At 268 and 536 mg/kg-day, lethargy and rough coats were reported (incidences not reported). Terminal body weights were slightly lower than controls in male mice receiving doses ≥268 mg/kg-day, but the differences from control were <10% (4−6%) and were not statistically significant. Female mice had >10% increases in absolute liver weight at doses greater than or equal to 536 mg/kg-day (13−22% compared to control) and in relative liver weight (11−28% compared to controls) at all doses (see Table B-2). Absolute liver-weight increases >10% were observed at doses ≥134 mg/kg-day, and relative liver-weight increases >10% were observed in the highest dose group (1,071 mg/kg-day) in males. No treatment-related gross or microscopic lesions were observed (data not shown). These data indicate that 536 mg/kg-day is a FEL for mortality (2/10 females died). Based on the >10% increases in relative liver weight in female mice at all doses, a LOAEL of 67 mg/kg-day (lowest dose tested) is identified for this study. No NOAEL is identified.

Chronic-Duration and Carcinogenicity Studies

NTP (1988)

A chronic toxicity and carcinogenicity study of MBT (purity 96−97%) was conducted in rats and mice (NTP, 1988). Groups of 50/sex F344/N rats and B6C3F$_1$ mice were administered MBT in corn oil by gavage at doses of 0, 188 (female rats only), 375, or 750 (male rats and both sexes of mice) mg/kg for 5 days/week for 103 weeks. The respective ADDs, calculated as the product of the gavage dose and 5/7 days per week, were 0, 134, 268, or 536 mg/kg-day. The animals were observed twice daily for mortality and moribundity, and clinical signs were recorded once a week. Body weights were obtained weekly for the first 12 weeks and monthly thereafter until study termination. Hematology, clinical chemistry, and organ weights were not evaluated. All animals, including those that were found dead, were evaluated for gross and microscopic pathology; tissues examined for histopathology included: adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), gall bladder (mice only), heart, kidneys, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate and testes or ovaries and uterus, salivary glands, small intestine, spleen, spinal cord, sternebrae or femur or vertebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Three statistical analyses were conducted, including life table analysis, incidental tumor analysis, and unadjusted analyses (Fisher's exact and Cochran-Armitage linear trend tests). Life table and incidental tumor analyses, which adjust for intercurrent mortality, were applied to the male rat data to account for survival differences among groups. Life table analysis assumes that all tumors observed in animals dying before the end of the study were "fatal," and incidental tumor analysis assumes that all tumors observed before the end of the study were "incidental" and unrelated to the cause of death.

A statistically significant (at both doses), dose-dependent decrease in survival was seen in treated male rats beginning in Week 83 (cumulative incidences of nonaccidental deaths prior to termination, including moribund sacrifices, were 8/50, 28/50, and 29/49 in control, low-dose, and high-dose males); survival of treated females did not differ from controls, but was low in all groups (nonaccidental deaths prior to termination were 21/49, 18/49, and 25/50 in control, low-dose, and high-dose females) (NTP, 1988). Deaths of female rats occurred throughout the study, while male deaths primarily occurred after at least 60 weeks on study. The study authors reviewed individual animal data and noted that most of the rats that died prematurely had tumors. Body weights of males and females were higher than (not statistically significant), or did not differ from, controls. The only clinical sign noted was lethargy after dosing; the study authors did not report incidences of this finding or any further details. Table B-3 shows selected

non-neoplastic effects. Statistically significantly increased incidences of non-neoplastic lesions in the forestomach (ulcers, inflammation, epithelial hyperplasia, and hyperkeratosis) were observed in males of both dose groups. Incidences of non-neoplastic forestomach lesions also were increased in treated females, although the incidences were lower than in males, and only the incidence of ulcers in the high-dose group was statistically significantly different from the control incidence (see Table B-3). NTP (1988) reported an increase in the mean severity score for nephropathy (observed in all male rats and >75% of female rats, including controls) in exposed male rats. Severity scores of 3.4 (moderate–severe) were recorded in male rats exposed to 268 and 536 mg/kg-day vs. 2.3 (mild–moderate) in control males. NTP (1988) also noted that renal pelvic epithelial cell hyperplasia and tubular cell hyperplasia were observed in treated male rats and not in controls, although the incidences (1−4 rats in groups of 49−50) were not statistically significantly different from controls. Determination of effect levels in this study is complicated by the statistically significant reductions in survival and statistically significantly increased incidences of multiple tumor types (discussed further below) at 268 and 536 mg/kg-day in male rats. In female rats, the high dose of 268 mg/kg-day might be considered a LOAEL based on an increased incidence of forestomach ulcers; however, because this dose was associated with reduced survival in males and tumors in both males and females, it cannot be used as the LOAEL. Although non-neoplastic effects were not seen in females at 134 mg/kg-day, this dose cannot be identified as a NOAEL due to the confounding effect of tumors seen at this dose.

Increased incidences of neoplastic lesions were reported in a number of rat tissues (see Table B-4). Pairwise comparisons by life table analysis showed statistically significantly increased incidences of adrenal gland pheochromocytoma (with or without malignant pheochromocytoma), pancreatic acinar cell adenoma, and preputial gland adenoma (and adenoma or carcinoma) in male rats at both doses. Pairwise comparisons by life table analysis showed statistically significantly increased incidences of mononuclear cell leukemia and pituitary gland adenoma in male rats at the low dose but not the high dose. At the high dose, the incidences of subcutaneous fibroma and subcutaneous fibroma, neurofibroma, sarcoma, or fibrosarcoma (combined) were statistically significantly increased in male rats when analyzed by life table test; however, NTP (1988) stated that the incidences were not statistically significant by the incidental tumor test. A statistically significant trend by life table or incidental tumor test was reported in male rats for incidence of mesotheliomas, pancreatic acinar cell adenoma, adrenal gland pheochromocytoma, preputial gland adenoma or carcinoma, and subcutaneous fibroma, neurofibroma, sarcoma, or fibrosarcoma (combined). NTP (1988) reported the incidence did not exceed the historical control range for corn oil vehicle animals for mesothelioma and preputial gland adenoma (and adenoma or carcinoma); however, these lesions were statistically significantly different from concurrent controls. In female rats, exposure to the high dose resulted in statistically significantly increased incidences of pituitary gland adenoma and adenoma or carcinoma (combined) and adrenal gland pheochromocytoma; pituitary and adrenal gland tumor incidences at the low dose were not statistically significantly elevated, but the incidences were very similar to the high-dose incidences (see Table B-4). NTP (1988) concluded that under the conditions of the rat study, there was *"some evidence of carcinogenic activity"* among male rats (based on increased incidences of mononuclear cell leukemia, pancreatic acinar cell adenomas, adrenal gland pheochromocytomas, and preputial gland adenomas or carcinomas), and *"some evidence of carcinogenic activity"* among female F344/N rats (based on increased incidences of adrenal gland pheochromocytomas and pituitary gland adenomas).

A total of six high-dose male mice and four high-dose females died during Week 13 after they were accidentally dosed twice in a 16-hour period; these animals were not included in the statistical analysis of survival after Week 12. After excluding the accidental deaths, analysis of survival among high-dose females showed statistically significantly reduced survival (22/46 survived to termination, compared with 30/44 controls, $p = 0.004$). The deaths of high-dose female mice occurred early in the study; the first high-dose female death occurred during Week 3 on study, and deaths began to occur regularly after Week 16. Statistical analysis of survival of treated male mice showed no difference from controls; however, Kaplan-Meier survival curves showed that a number of high-dose male mice died early in the study (there were 10 nonaccidental deaths between Weeks 7 and 47). After the first year, survival of high-dose males stabilized such that survival to termination was not statistically different from controls. NTP (1988) reported that lung hemorrhage and congestion were seen in many of the mice that died prematurely and that tumors were not seen in these animals. Table B-5 summarizes the incidences of nonaccidental deaths in male and female mice. As with the rats, mice were observed to be lethargic after dosing (incidence of this effect not reported). NTP (1988) observed dose-dependent decreases (4−14% lower than controls) in mean body weights among male mice between Weeks 3 and 64; subsequently, dosed males regained body weight such that terminal body weights were comparable to controls. Among females, mean body weights of the high-dose animals were lower than controls between Weeks 42 and 90, but the difference did not exceed 6%. Mean body weights of the low-dose females were comparable to those of controls throughout the study period. Minimal to mild bronchopneumonia occurred in all groups of mice, including controls, at incidences between 24 and 49%; this effect was attributed to Sendai virus infection based on serology in sentinel animals. Based on the minimal to mild severity of the bronchopneumonia, it is unlikely that the viral infection contributed to the early deaths, although individual animal data on this endpoint are not available to investigate this relationship or the potential relationship between the bronchopneumonia and the lung hemorrhage and congestion. No treatment-related non-neoplastic lesions were seen in males or females at any dose. The high dose in this study (536 mg/kg-day) is considered to be a FEL based on decreased survival of mice beginning early in the study and in the absence of tumors. As discussed further below, the low dose (268 mg/kg-day) was associated with an increased incidence of hepatocellular adenomas in female mice and thus cannot be considered a NOAEL, despite the lack of non-neoplastic effects.

Despite the early mortality in high-dose mice, NTP (1988) concluded that the final survival rates were sufficient to allow evaluation of potential carcinogenicity. No evidence of treatment-related neoplastic lesions were seen in male mice. In low-dose females, the incidence of hepatocellular adenoma or carcinoma was statistically significantly increased; however, the incidence at the high dose was not different from controls (see Table B-5). NTP (1988) noted that the absence of tumors at the high dose may have been due to the decreased survival in this group, as the premature deaths occurred early in the study, and hepatocellular tumors tend to appear late in mice. NTP (1988) concluded that, under the conditions of the study, there was *"no evidence of carcinogenic activity"* in male $B6C3F_1$ mice, but *"equivocal evidence of carcinogenic activity"* in female $B6C3F_1$ mice based on the increased incidence of hepatocellular adenomas or carcinomas (combined).

Garcia (2004); Ogawa et al. (1989)

Ogawa et al. (1989) conducted a 20-month chronic toxicity study of MBT (purity unknown) in mice (Slc:ddY), which was published in Japanese. Only the abstract from Ogawa

et al. (1989) was available in English and a summary of the study was published by Garcia (2004). The information below comes from the Ogawa et al. (1989) abstract and the summary by Garcia (2004). Groups of 30 mice per sex (treated) and 60 control mice per sex received 0, 30, 120, 480, or 1,920 ppm MBT in the diet for 20 months. Garcia (2004) calculated daily doses of 0, 3.60, 14.69, 57.90, or 289.40 mg/kg-day, respectively, in males and 0, 3.61, 13.52, 58.82, or 247.98 mg/kg-day, respectively, in females. Interim sacrifices were conducted at 6 and 12 months, leaving groups of 6−14 male mice and 7−15 female mice for sacrifice and evaluation at the end of the 20-month exposure. Based on information provided by Garcia (2004), toxicological evaluations in the study included clinical signs, body weight, food consumption, hematology (red blood cell [RBC] and white blood cell [WBC] counts, hemoglobin [Hb], hematocrit [Hct], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and platelets), serum chemistry (total protein, albumin, albumin:globulin ratio, blood urea nitrogen [BUN], total cholesterol, alkaline phosphatase [ALP], alanine aminotransferase [ALT], and aspartate aminotransferase [AST]), and histopathology of the lungs, liver, and kidneys.

Garcia (2004) did not discuss mortality in the summary of Ogawa et al. (1989). Based on the review, decreased body-weight gain was observed in highest-dose males throughout the study. No treatment-related effects were seen in hematological parameters (Garcia, 2004). An increased rate of cell infiltration in the interstitium of the kidneys (cell type not provided) was reported in male mice at 57.90 and 289.40 mg/kg-day at the end of the study; however, this observation may reflect a spurious finding because (1) there was no clear dose-response at the 12-month sacrifice; (2) the incidences did not increase (and at some doses, actually decreased) between the 12- and 20-month sacrifices; (3) the numbers of animals per group were small (5−10) at each sacrifice; and (4) control animals had highly variable incidence of interstitial cell infiltration of the kidneys (7−60%), suggesting high incidence of spontaneous lesions. No treatment-related increase in non-neoplastic histopathology of the lung or liver, nor tumor incidence in the lung, liver, or kidneys was seen. Effect levels for this study cannot be identified because available information is limited to an abstract and a secondary report.

Innes et al. (1969)

In a study examining the potential carcinogenicity of 120 compounds, two hybrid strains of mice (C57BL/6 × C3H/Anf, strain "X" and C57BL/6 × AKR, strain "Y") were given MBT (purity not specified; Captax formulation) via gavage (in 0.5% gelatin) at the estimated maximum tolerated dose (MTD) of 100 mg/kg from Postnatal Day (PND) 7 to weaning at 4 weeks of age. The daily dose was not adjusted for weight gain during the gavage exposure period. From these animals, groups of 18 mice/sex/dose were selected to continue exposure to MBT in the diet for 17 additional months (total exposure duration 18 months) at a concentration of 323 ppm; this concentration was selected to yield the same 100-mg/kg dose based on food intake and body weight at 4 weeks of age. A separate group of mice received zinc mercaptobenzothiazole (Zetax) at 1,000 mg/kg in 0.5% gelatin until 4 weeks of age and then 3,385 ppm zinc MBT in the diet for 17 months. Crude estimates of the time-weighted average

(TWA) daily doses[4] using default food intake and body weight from chronic-duration studies are 57.4 mg/kg-day for males and 57.7 mg/kg-day for females for MBT administration and 252 mg/kg-day for males and 253 mg/kg-day for females (as equivalent dose of MBT) for zinc MBT administration. Human equivalent doses (HEDs) of 8.04 and 8.08 mg/kg-day were calculated for male and female mice exposed to MBT from these dose estimates using the mouse:human dosimetric adjustment factor (DAF) of 0.14 based on the animal:human body-weight ($BW^{1/4}$) ratio recommended by U.S. EPA (2011b). HEDs of 35.3 and 35.4 mg/kg-day are calculated for male and female mice administered zinc MBT. All animals were sacrificed at ~18 months of age. The study authors reported no statistically significant increase in tumor incidences in either the MBT or the zinc MBT groups, but quantitative results were not given.

Reproductive Studies

Springborn Laboratories (1990b)

In a two-generation reproduction toxicity study, Springborn Laboratories (1990b) administered MBT (98.5 and 98.2% purity; no further information regarding impurities was reported) in the diet to groups of 28 male and female S-D (Crl:CD®COBS®BR) rats at concentrations of 0, 2,500, 8,750, or 15,000 ppm for at least 70 days prior to cohabitation, during cohabitation, and until scheduled sacrifice. ADDs estimated from default body weight and food intake values for S-D rats in a chronic-duration study (U.S. EPA, 1988) are 0, 172.1, 602.3, or 1,033 mg/kg-day for males and 0, 199.7, 699.0, or 1,198 mg/kg-day for females, respectively.[5] After birth, litters (F1) were raised until Lactation Day (LD) 21, when selected weanlings were administered MBT in the diet at the same doses as their parents. F0 and F1 parents were observed for clinical signs of toxicity, morbidity, or mortality daily. Body weights and food intake were measured weekly for F0 and F1 parental males for the duration of the study, and for females prior to copulation and at intervals during gestation and lactation. Parameters related to reproductive function, including precoital interval, copulation, fertility indices, pregnancy percentage, and gestation length, were evaluated. F0 and F1 parents were sacrificed and necropsied after weaning of their offspring (F1 and F2, respectively). The liver, kidneys, and testes or ovaries of all F0 and F1 parents were weighed. Selected tissues and organs of the F0 and F1 parents were examined microscopically in the control and high-dose groups (1,033 or 1,198 mg/kg-day). In addition, microscopic examinations of the kidneys of F0 parents of the low and mid-range doses, and the kidneys and livers of F1 parents of the low and mid doses were performed. Offspring viability was determined daily in both the F1 and F2 generations. The study authors examined F1 and F2 offspring on LDs 0, 4, 7, 14, and 21 and recorded body weights on LDs 1, 4, 7, 14, and 21. On LD 4, litter size was reduced to eight offspring (four each males and females, if possible). Moribund and culled pups were sacrificed and necropsied, and the surviving F2 offspring were sacrificed and necropsied on LD 21. Statistical analysis of

[4]For each compound, the dose administered by gavage is assumed to remain constant during the 3-week administration period. The dietary concentration is converted to an ADD using male and female default values for food intake and body weight in $B6C3F_1$ mice (data on the tested strains were not available) in chronic-duration studies (U.S. EPA, 1988). The doses administered by gavage and diet are time weighted (3 weeks via gavage and 75 weeks via diet) to yield the estimated ADDs. The equivalent dose of MBT is calculated from the dose of zinc MBT by multiplying by the ratio of the molecular weights of the two compounds (167.24 g/mol:397.7 g/mol).

[5]Body weight and food consumption data are not provided in the study report. The ADDs are calculated using default values for food intake and body weight in male and female S-D rats in chronic-duration studies (U.S. EPA, 1988). For example, 2,500 ppm MBT = 2,500 mg/kg MBT × (0.036 kg/day ÷ 0.523 kg) = 172.1 mg/kg-day.

continuous data employed ANOVA and Dunnett's test; pup sex ratios and pup viability were evaluated using the χ^2 test.

Survival of F0 and F1 parents was not affected by exposure to MBT (Springborn Laboratories, 1990b). Clinical signs, litter sizes, and pup viability were comparable for control and treated animals of all generations. Metrics of reproductive function were also similar in the control and treated animals in both generations. Body weight was decreased in several treated groups but the change was <10% compared to controls. In F0 and F1 parental males, body weights were statistically significantly lower than controls at 602.3 and 1,033 mg/kg-day throughout most of the study; at these doses, statistically significant decreases of 6−9% were recorded at the last measurement. High-dose F0 females also exhibited statistically significantly decreased body weight, accompanied frequently by decreased food intake, from Week 3 through the premating period, on Gestation Day (GD) 20, on LDs 1 and 14, and during Weeks 19 and 20. At 699.0 mg/kg-day, female F0 rats exhibited sporadic decreases in body weight during the premating period and Week 20. Terminal body weights in F0 females were statistically significantly lower than controls at 699.0 and 1,198 mg/kg-day (5 and 7% respectively). In F1 females, statistically significant reductions in body weight were seen consistently at all doses in the premating period and in the mid- and high-dose groups during gestation and lactation; terminal body weights were statistically significantly lower only at the mid and high doses (6−7%). Statistically significantly decreased food intake was noted occasionally in F0 and F1 males and females at ≥602.3 or 699.0 mg/kg-day.

Necropsy body weights were not reported; as noted earlier, terminal body weights (Week 20 in F0 animals and Week 38 in F1 animals) were statistically significantly reduced (5−9% lower than controls) in both F0 and F1 male and female parents exposed to 602.3 or 699.0 or 1,033 or 1,198 mg/kg-day (Springborn Laboratories, 1990b) (see Table B-6). In F0 male and female parents, relative liver weights were statistically and biologically significantly increased at 602.3 or 699.0 and 1,033 or 1,198 mg/kg-day (12−17% compared with controls), while absolute liver weights were increased by ≤7% compared with controls (not statistically significantly), suggesting that the relative weight change may reflect body-weight reductions. In F1 male and female parents, statistically and biologically significant increases in relative liver weight at the mid and high doses (15−33%) were accompanied by absolute liver-weight increases of 8−22%, suggesting that in these animals, the relative weights were not primarily attributable to body-weight reductions. F1 male parents also exhibited statistically and biologically significantly increased relative liver weight (12%) accompanied by an absolute liver-weight increase of 9% at the low dose of 172.1 mg/kg-day. The liver-weight changes are consistent with the histopathology findings of statistically significantly increased incidences of hepatocyte hypertrophy in male and female F1 parents at 602.3 or 699.0 and 1,033 or 1,198 mg/kg-day, with greater incidences in males (see Table B-7).

In male F0 and F1 parents, relative kidney weights were statistically significantly increased at 602.3 and 1,033 mg/kg-day (15−20%), and absolute kidney weights were increased by 8−10% at these doses (statistically significant only in high-dose F1 males; see Table B-6). Relative kidney weights were increased ≥10% in the mid- and high-dose groups of F0 and F1 female parents (10−15%); these changes were accompanied by increases in absolute kidney weight of 3−7% and were likely influenced by body-weight reductions. Treatment-related histopathological changes were observed in kidneys (primarily in males) of F0 and F1 parental rats. Statistically significantly increased incidences of cortical tubular basophilia were observed

at all doses in F0 males and at 1,033 mg/kg-day in F1 males, but not in females (see Table B-7). Statistically significantly increased incidences of brown pigment in the lumen and epithelial cells of the proximal convoluted tubules were observed at 602.3 and 1,033 mg/kg-day in F0 and F1 males and at 1,198 mg/kg-day in F1 females; the study authors suggested that the presence of brown pigment in the lumen reflected excretion via the kidney rather than a toxic effect on the kidney, but provided no information to support this hypothesis. The study authors also reported increased incidences of alpha 2u-globulin (α2u-g) inclusions in epithelial cells of the proximal convoluted tubules in male rats. However, the study authors' conclusion was based on hematoxylin-eosin staining rather than immunohistochemistry, which provides more rigorous evidence; further, there were no other signs of α2u-g accumulation (e.g., granular casts, exfoliation of cells into the tubular lumen, increased mitotic figures) (Frazier et al., 2012; U.S. EPA, 1991).

Body weights of F1 pups were statistically significantly reduced at 602.3 or 699.0 and 1,033 or 1,198 mg/kg-day on LD 14 (8 and 14% lower than controls, respectively) and LD 21 (12 and 21%, respectively; see Table B-8). In F2 pups, statistically significant body-weight reductions were observed at all doses on LD 14 (9, 9, and 14% lower than controls in the low-, mid-, and high-dose groups) and LD 21 (9, 12, and 21% lower than controls). A LOAEL of 172.1 mg/kg-day (the lowest dose tested) is identified based on increased incidence of basophilic tubules in male F0 rats and a >10% increase in relative liver weight in male F1 rats.

Developmental Studies

Springborn Laboratories (1989c)

Springborn Laboratories (1989c) conducted a dose range-finding study for a developmental study in groups of timed-mated female S-D rats (six/group) exposed to MBT (98% purity) suspended in corn oil via gavage at doses of 0, 300, 600, 1,000, 1,500, or 2,200 mg/kg-day once daily on GDs 6−15. The control group received the vehicle only. During the study, maternal animals were examined for mortality twice daily and for clinical signs of toxicity once daily. Maternal body weight was measured on GDs 0, 6, 9, 12, 16, and 20. Maternal body-weight gain was determined for GDs 0−6, 6−9, 9−12, 12−16, 16−20, 6−16, and 0−20. All surviving dams were sacrificed on GD 20, and cesarean sections were performed. A maternal necropsy was performed that included examination of external surfaces, orifices, and viscera, and determination of pregnancy status. The number of viable and nonviable fetuses, number of early and late resorptions, and number of corpora lutea were recorded. The fetuses were weighed, sexed, and examined externally for morphological abnormalities.

Decreased survival was reported following exposure to 2,200 mg/kg-day. Two rats died before GD 20 (on GD 10 and GD 11), and lesions were noted in their gastrointestinal tracts. Clinical signs of toxicity were reported in rats of all dose groups and included urine staining, dark material around eyes, nose, and mouth, salivation, and chin dragging. During GDs 6−9, maternal body-weight gain was decreased in rats exposed to 1,500 and 2,200 mg/kg-day (6−19 g loss compared with 3 g gain in controls), but were not statistically different than control by the end of gestation (≤4% decrease from control). No differences were observed between the control and treatment groups for the other maternal and developmental toxicity parameters examined. Due to excessive maternal mortality at 2,200 mg/kg-day, Springborn Laboratories (1989c) chose dosage levels of 300, 1,200, and 1,800 mg/kg-day for the subsequent teratology study (Springborn Laboratories, 1989e). Based on these data, 2,200 mg/kg-day is a FEL for mortality (2/6 rats died).

Springborn Laboratories (1989b)

Springborn Laboratories (1989b) conducted a dose range-finding study for a developmental study in groups of artificially inseminated New Zealand white (NZW) rabbits (five/group) were exposed to MBT (98% purity) suspended in 1% methylcellulose via gavage at doses of 0, 150, 300, 600, 1,000, or 1,500 mg/kg-day once daily on GDs 6−18. During the study, maternal animals were examined for mortality twice daily and for clinical signs of toxicity once daily. Maternal body weight was measured on GDs 0, 6, 9, 12, 15, 19, 24, and 29. Maternal body-weight gain was determined for GDs 0−6, 6−9, 9−12, 12−15, 19−24, 24−29, 6−19, 0−29, and 19−29. All surviving does were sacrificed on GD 29, and cesarean sections were performed. Maternal necropsy was performed on animals that died prior to GD 29. The number of viable and nonviable fetuses, number of early and late resorptions, and number of corpora lutea were recorded. The fetuses were weighed and examined externally for morphological abnormalities.

Decreased survival was reported at doses ≥600 mg/kg-day (1/5 deaths on GD 27 at 600 mg/kg-day, 3/5 deaths on GDs 17−20 at 1,000 mg/kg-day, 5/5 deaths on GDs 11−12 at 1,500 mg/kg-day). Abortions were reported in one rabbit in each of the 600- and 1,000-mg/kg-day groups on GDs 27 and 14, respectively. None of the rabbits in the 1,000- and 1,500-mg/kg-day groups survived until scheduled cesarean section. Clinical signs of toxicity were reported at all treatment levels, including reduced defecation, emaciation (≥600 mg/kg-day), and labored breathing (1,500 mg/kg-day). Terminal body-weight decreased >10% in does administered ≥600 mg/kg-day, with dose-dependent decreases in body weight in all treatment groups. Body-weight gain was less than controls in all treatment groups throughout the majority of gestation but was comparable to controls in the 150- and 300-mg/kg-day groups at end of gestation (GDs 19−29). Two does administered 300 mg/kg-day had severe body-weight losses during gestation. Intrauterine survival was decreased in the 600-mg/kg-day group due to increased early and late resorptions and postimplantation loss. Fetal weights were dose-dependently decreased >10% in all treatment groups (−14, −16, and −61% in 150, 300, and 600 mg/kg-day, respectively). No biologically significant differences in fetal external abnormalities were observed between the control and treatment groups examined. Due to excessive maternal and developmental toxicity at 600 mg/kg-day, Springborn Laboratories (1989b) chose doses of 50, 150, and 300 mg/kg-day for the subsequent teratology study. Based on decreased fetal weight, a developmental LOAEL (lowest dose tested) of 150 mg/kg-day is identified. Based on increased maternal mortality, 600 mg/kg-day is identified as a FEL (1/5 rabbits died).

Springborn Laboratories (1989e)

Springborn Laboratories (1989e) evaluated teratogenic effects of MBT in groups of timed-mated female S-D rats (26/group) exposed to MBT (98% purity) suspended in corn oil via gavage at doses of 0, 300, 1,200, and 1,800 mg/kg-day once daily on GDs 6−15. The control group received the vehicle only. Dose estimates were calculated by the study authors using the mean body weight of vehicle control animals on GD 0 (i.e., 0.281 kg). Analytical measurement indicated that dosing formulations were within ±10% of nominal concentrations. During the study, maternal animals were examined for mortality twice daily and for clinical signs of toxicity once daily. Maternal body weight and food consumption were measured on GDs 0, 6, 9, 12, 16, and 20. All surviving dams were sacrificed on GD 20, and cesarean sections were performed. Maternal necropsy examinations included an evaluation of external surfaces, orifices, and viscera, and a thorough uterine examination. The number of viable and nonviable fetuses, number of early and late resorptions, and number of corpora lutea were recorded. Uteri with no

evidence of implants were examined macroscopically for the detection of early embryonic deaths. The fetuses were weighed, sexed, and examined externally for morphological abnormalities. Approximately one-half of the fetuses were examined for visceral abnormalities and one-half were examined for skeletal abnormalities. Statistical analyses of continuous endpoints was done using ANOVA followed by Dunnett's test. The Mann-Whitney U test was used to assess postimplantation losses and numbers of dead fetuses and resorptions, while the χ^2 test was used for sex ratio. Incidences of malformations and variations were evaluated using Fisher's exact test. Developmental endpoints were evaluated using both fetuses and litters as the experimental units of analysis.

No treatment-related maternal mortalities were observed (Springborn Laboratories, 1989e). One maternal death occurred in the 1,800-mg/kg-day group on GD 16 and was attributed to a congenital anomaly (umbilical hernia), which was aggravated by pregnancy and not considered to be treatment-related. Maternal survival was 100% in the other dose groups. Clinical signs of toxicity in maternal animals were seen at the 1,200- and 1,800-mg/kg-day doses (see Table B-9). The most prevalent signs were seen postdosing, and included salivation, dark material around the mouth, and urine staining. A decrease in activity was also observed postdosing in several rats administered 1,800 mg/kg-day. During GDs 6−9, body-weight gain of maternal animals at 1,800 mg/kg-day was statistically significantly decreased (loss of 9 g compared with gain of 3 g in controls) and food consumption was decreased by 27% relative to controls during this time. However, the decrease in mean maternal body weight on GD 9 was small (4% less than controls at the high dose). After GD 9, increased food consumption was seen at 1,200 and 1,800 mg/kg-day (11 and 14% higher than controls, respectively, during GDs 9−12), and mean body weights of treated rats were not statistically or biologically significantly different from controls (see Table B-10).

Springborn Laboratories (1989e) reported pregnancy percentages of 92.3, 88.5, 96.2, and 84.6% at 0, 300, 1,200, and 1,800 mg/kg-day, respectively. Mean postimplantation loss per litter was increased, primarily due to increases in early resorptions, at ≥300 mg/kg-day (see Table B-11). Springborn Laboratories (1989e) reported that the mean numbers of postimplantation losses per litter were within the laboratory historical control range (range: 0.6−1.4; mean: 0.9) for rats of the same strain at 300 (mean of 1.3 per litter) and 1,200 (1.3 per litter) mg/kg-day, but was well above the range at 1,800 mg/kg-day (1.7 per litter). However, the concurrent controls did not exhibit any abnormal toxicity and were used for comparison. No statistically significant or biologically significant differences were observed between the control and treatment groups for the other maternal and developmental toxicity parameters examined. EPA identifies a maternal LOAEL of 1,800 mg/kg-day and a NOAEL of 1,200 mg/kg-day, based on clinical signs of toxicity and decreased activity. Based on increased postimplantation loss compared to the concurrent control group, the developmental LOAEL (lowest dose tested) is 300 mg/kg-day.

Springborn Laboratories (1989d)

Springborn Laboratories (1989d) also evaluated developmental toxicity of MBT in rabbits. Artificially inseminated NZW rabbits (20/group) were exposed to MBT (98% purity) suspended in 1% methylcellulose via gavage at doses of 0, 50, 150, or 300 mg/kg-day once daily on GDs 6−18. The control group received the vehicle only. Dose estimates were calculated using the mean body weight of vehicle control animals on GD 0 (i.e., 4.413 kg). Analytical measurement indicated that dosing formulations were within ±8% of nominal concentrations.

During the study, maternal animals were examined for mortality twice daily and for clinical signs of toxicity once daily. Maternal body weight was measured on GDs 0, 6, 9, 12, 15, 19, 24, and 29, and food consumption was measured daily. Maternal body-weight gain and food consumption (calculated as g/animal-day and g/kg-day) were determined for GDs 0−6, 6−9, 9−12, 12−15, 15−19, 19−24, 24−29, 6−19, and 0−29. All does were sacrificed on GD 29, and cesarean sections were performed. For maternal necropsy examinations, the thoracic, abdominal, and pelvic cavities were opened, the viscera examined, and the livers weighed. The numbers of viable and nonviable fetuses, early and late resorptions, and corpora lutea were recorded. Uteri with no macroscopic evidence of implants were examined for the detection of early embryonic deaths. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities. Statistical analyses of continuous endpoints were done using ANOVA followed by Dunnett's test. The Mann Whitney U test was used to assess postimplantation losses and numbers of dead fetuses and resorptions, while the χ^2 test was used for sex ratio. Incidences of malformations and variations were evaluated using Fisher's exact test. Developmental endpoints were evaluated using both fetuses and litters as the experimental units of analysis.

There were no treatment-related maternal mortalities or clinical signs of toxicity among the rabbits (Springborn Laboratories, 1989d). One doe in the 150-mg/kg-day group died on GD 13 due to trauma associated with intubation. During GDs 15−19, a statistically nonsignificant reduction in maternal body-weight gain occurred at 300 mg/kg-day (<10% relative to the control group). No other effects on body weight nor effects on food consumption, were observed. At necropsy, no macroscopic abnormalities were observed that were considered to be test substance-related. Statistically nonsignificant increases in absolute liver weights occurred at 150 and 300 mg/kg-day (4−7% higher than controls) and relative liver weight at 300 mg/kg-day (4% higher than control). No statistically significant or biologically relevant differences were observed between the control and treatment groups for any of the other maternal or developmental toxicity parameters examined. Given the small magnitude of changes in body weight and liver weight at 300 mg/kg-day, these effects are not considered as the basis for determining a LOAEL. Thus, the highest dose tested of 300 mg/kg-day is identified as the NOAEL for maternal and developmental toxicity.

Inhalation Exposures

No studies evaluating the effects of MBT in animals after repeated inhalation exposure have been located in the available literature.

OTHER DATA (GENOTOXICITY, ACUTE TESTS, OTHER EXAMINATIONS)

Table 4 provides an overview of genotoxicity studies of MBT. Other supporting studies on MBT are discussed afterward, including:

- acute-duration oral and inhalation studies,
- studies using other routes of exposure,
- an acute immunotoxicity study,
- an acute neurotoxicity study, and
- metabolism/toxicokinetic studies.

Table 4. Summary of MBT Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Genotoxicity studies in prokaryotic organisms						
Mutation	*Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538	3, 10, 30, 100, 300 µg/plate; Second test with TA98 and TA1538 at 100, 250, 300, 450, 600 µg/plate	–	–	Plate incorporation assay. Toxicity observed at doses ≥333 µg/plate.	Pharmakon Research International (1984a, 1984b)
Mutation	*Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538	0.1, 1.0, 10, 100, 500 µg/plate	–	–	Plate incorporation assay.	Litton Bionetics (1976)
Mutation	*Salmonella* strains TA98, TA100, TA1535, and TA1537	0, 3.3, 10, 33, 100, 333, 1,000, 3,333, 10,000 µg/plate (TA98 also tested at 200, 400, 500, 600, 700, 10,000 µg/plate)	–	–	Plate incorporation assay. Strain TA98 had equivocal and weakly positive results at doses ≥333 µg/plate in some trials with metabolic activation, but other trials at same doses were negative. In addition, slight toxicity seen at doses ≥333 µg/plate; precipitation seen at ≥3,333 µg/plate.	NTP (1988); Zeiger et al. (1987)
Mutation	*Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538	Without activation: 32, 100, 320, 500, 1,000 µg/plate With activation: 100 µg/plate	–	–	These data provided in a handwritten report without any information on methods.	Goodyear Tire & Rubber Company (1985)
Mutation	*Salmonella* strains TA98, TA100, TA1535, and TA1537	0.1, 1, 10, 100 µg/plate	–	–	Plate incorporation assay. Toxicity was observed at 1,000 µg/plate.	Goodyear Tire & Rubber Company (1985)

Table 4. Summary of MBT Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*Salmonella* strains TA98, TA100, TA1535, and TA1537	8−200 µg/plate	NT	−	Plate incorporation assay.	Crebelli et al. (1985)
Mutation	*Salmonella* (strains not reported)	NR	−	−	Study details not provided; MBT exhibited a statistically nonsignificant mutagenic response at nontoxic concentrations (toxic concentration not reached). It is unclear whether results were with or without metabolic activation.	Donner et al. (1983)
DNA damage (SOS/umu)	*Salmonella* strain TA1535/pSK1002	Without S9: up to 19 µg/mL (LC_{50}) With S9: up to 20 µg/mL (LC_{50})	−	−	MBT did not induce DNA damage at concentrations less than or equal to the LC_{50} with or without metabolic activation; higher concentrations were not tested.	Ye et al. (2014)
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	*Saccharomyces cerevisiae* strain D4	0.1, 1.0, 10, 100, 500 µg/plate	−	−	Plate incorporation assay.	Litton Bionetics (1976)
Genotoxicity studies in mammalian cells in vitro						
Mutation	Mouse lymphoma cells (L5178Y)	Without S9: 0, 3.75, 15, 40, 60, 80, 100, 150 µg/mL With S9: 0, 3.75, 5, 15, 20, 40, 50, 60, 80, 100, 120, 150 µg/mL	±	±	MBT was mutagenic only at concentrations that were highly cytotoxic with or without metabolic activation. Mutant frequency was increased 1.8−8.7-fold without metabolic activation (at <10% relative survival) and 1.7−2.7-fold with metabolic activation (at 7−20% relative survival). Cytotoxicity (survival <50% of controls) was observed at ≥3.75 µg/mL without metabolic activation and ≥20 µg/mL with metabolic activation. Observed effects are likely due to cytotoxicity rather than genotoxicity.	Litton Bionetics (1985)

Table 4. Summary of MBT Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	Mouse lymphoma cells (L5178Y)	Without S9: 30, 40, 50, 60, 80, 100, 120, 150 µg/mL With S9: 1.25, 2.50, 4, 5, 6, 7.5, 8, 10, 12, 15, 16, 20 µg/mL	−	+	Mutagenic with metabolic activation at ≥5 µg/mL. Cytotoxicity (survival <50% of controls) was observed at ≥40 µg/mL without metabolic activation and at ≥7.5 µg/mL with metabolic activation.	NTP (1988)
Mutation	Mouse lymphoma cells (L5178Y)	Without S9: 0, 30, 40, 50, 60, 80, 100, 150 µg/mL With S9: 0, 4, 5, 6, 8, 10, 12, 16, 20 µg/mL	−	+	A statistically significant 2−3-fold increase in mutant frequency was observed at ≥5 µg/mL with metabolic activation. Cytotoxicity (survival <50% of controls) was observed at ≥40 µg/mL without metabolic activation and at ≥8 µg/mL with metabolic activation.	Myhr et al. (1990)
Mutation (HGPRT)	CHO cells	Without S9: 1, 5, 10, 30, 50 µg/mL With S9: 10, 25, 75, 150, 300 µg/mL	−	−	Cytotoxicity was observed at doses ≥100 µg/mL without activation and at 1,000 µg/mL with activation; at 333.33 µg/mL with activation, survival relative to controls was 17%.	Pharmakon Research International (1984c)
Mutation (HGPRT)	Chinese hamster V79 cells	0, 50, 100, 300 µg/mL	−	NT	MBT was not cytotoxic at the highest concentration.	Donner et al. (1983)
SCE	CHO cells	Without S9: 0, 12.5, 14.9, 20.1, 24.8 µg/mL With S9: 0, 99.2, 247.5, 351.6, 401.6, 445.3, 501.5, 502.3, 750 µg/mL	−	+	A statistically significant 23−37% increase in SCEs was observed after exposure to ≥351.6 µg/mL in the presence of metabolic activation; SCEs were not induced without metabolic activation. No cells survived at concentrations of 24.8 µg/mL without metabolic activation or ≥502.3 µg/mL with metabolic activation.	NTP (1988)

Table 4. Summary of MBT Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
CA	CHO cells	Without S9: 0, 10, 14.9, 19.9, 30.1 µg/mL With S9: 0, 351.8, 373.5, 399, 400.8, 425, 450, 451 µg/mL	−	+	The percentage of cells with CAs was statistically significantly increased by 8−26% at ≥351.8 µg/mL with metabolic activation; CAs were not induced without metabolic activation. No cells survived at highest concentrations evaluated (30.1 µg/mL without metabolic activation, 500.5 µg/mL with metabolic activation in Trial 1, 450 µg/mL with metabolic activation in Trial 2).	NTP (1988)
CA	Chinese hamster lung cells	Without S9: 0, 200, 400 µg/mL With S9: 0, 200, 400, 600 µg/mL	−	±	Results with metabolic activation were judged as inconclusive at 400 µg/mL (5−10% total CA frequency). Results without metabolic activation were judged as negative (<5% total CA frequency). Cytotoxicity was observed at 400 µg/mL without activation and 600 µg/mL with S9 activation.	Matsuoka et al. (2005)
Micronucleus test	Human A549 lung carcinoma cells	0, 3.13, 6.25, 12.5, 25, 50, 100 µg/mL	−	NT	Cytotoxicity at the highest tested concentration was 15%.	Ye et al. (2014)
Micronucleus test	Human MGC-803 gastric carcinoma epithelial cells	0, 3.13, 6.25, 12.5, 25, 50, 100 µg/mL	−	NT	Cytotoxicity at the highest tested concentration was 29%.	Ye et al. (2014)
Cell transformation	Balb/3T3 cells	10.5−63 µg/mL	−	NT	Cytotoxicity (survival <50% of controls) was observed at doses ≥31.3 µg/mL.	Goodyear Tire & Rubber Company (1985)

Table 4. Summary of MBT Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Genotoxicity studies—in vivo						
Dominant lethal mutagenicity	S-D rats (28 M/group), dosed in diet for 13 wk, then mated for 2 wk to untreated females; females sacrificed on GD 13 for evaluation of numbers of embryonic deaths and viable embryos	0, 2,500, 8,750, 15,000 ppm in diet (0, 220, 770, 1,300 mg/kg-d)	−	−	No statistically significant or dose-related increase in embryonic deaths (no dominant lethal effect). In treated males, statistically significantly reduced food consumption and body weights were seen at ≥8,750 ppm. Significantly reduced body-weight gain occurred at 2,500 ppm during Wk 1−3 and 7−8.	Springborn Laboratories (1989a)
Mouse bone marrow CA assay	Swiss albino mice (four/group; sex not reported) were administered a single i.p. injection of zinc MBT in cotton seed oil and sacrificed 36 hr later for evaluation of CAs in bone marrow	0, 480, 960, 1,920 µg zinc MBT/20 g (24, 48, 96 mg/kg)	−	−	CA incidence was comparable between mice exposed to MBT and controls.	Mohanan et al. (2000)

Table 4. Summary of MBT Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
MN	CD-1 mice (four/sex/group) were administered a single i.p. injection of MBT and sacrificed 30 or 48 hr later. Additional groups (four/sex/group) were given two injections at 0 and 24 hr, and sacrificed 48 or 72 hr later; bone marrow was extracted and scored for polychromatic erythrocytes with MN	300 mg/kg per injection	−	−	No statistically significant increase in MN was seen in any group.	Pharmakon Research International (1984d, 1984e)

Table 4. Summary of MBT Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
DNA binding	F344 rats (10/sex) were given a single gavage dose of [2-^{14}C]-labelled MBT and sacrificed 8 hr later; radioactivity was measured in DNA collected from various tissues	375 mg/kg	−	−	Little radioactivity was detected in tissues; <0.6% in liver and <0.03% in remaining tissues. Based on radioactivity in DNA, low binding to DNA was measured: 1.5−3.0 pmol MBT/mg DNA in the liver and 0.15−0.76 pmol MBT/mg DNA in bone marrow. No DNA binding was detected in pancreatic, adrenal, or pituitary tissue. Covalent binding index (calculated as µmol chemical bound/mol DNA) ÷ (mmol chemical applied/kg body weight) was very low (1−3 in the liver; compared with a covalent binding index value of 6,000 for strong hepatocarcinogen, dimethylnitrosamine).	Brewster et al. (1989); Monsanto (1989a)

[a]+ = positive; − = negative; ± = inconclusive.

CA = chromosomal aberrations; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; GD = Gestation Day; i.p. = intraperitoneal; LC_{50} = median lethal concentration; M = male(s); MBT = 2-mercaptobenzothiazole; MN = micronuclei; NR = not reported; NT = not tested; S-D = Sprague-Dawley; SCE = sister chromatid exchange.

Genotoxicity

The potential genotoxicity of MBT has been evaluated in several in vitro studies and a limited number of in vivo studies. In general, the data indicate that MBT is not mutagenic. Limited in vitro data suggest that MBT may cause clastogenic effects in mammalian cells; however, findings are inconsistent among studies and cell types, and in vivo data were negative.

MBT was not mutagenic in *Salmonella typhimurium* strains with or without metabolic activation (Ye et al., 2014; NTP, 1988; Zeiger et al., 1987; Crebelli et al., 1985; Goodyear Tire & Rubber Company, 1985; Pharmakon Research International, 1984a, b; Donner et al., 1983; Litton Bionetics, 1976) or *Saccharomyces cerevisiae* strain D4 (Litton Bionetics, 1976). Urine obtained from 72 male workers employed in a tire factory who were exposed to MBT (along with numerous other chemicals) was also not mutagenic in *S. typhimurium* (Crebelli et al., 1985). In mammalian cells, MBT was mutagenic in mouse L5178Y lymphoma cells, but typically only at concentrations associated with cytotoxicity (Myhr et al., 1990; NTP, 1988; Litton Bionetics, 1985). MBT was not mutagenic in Chinese hamster ovary (CHO) cells with or without metabolic activation or Chinese hamster V79 cells without metabolic activation (Pharmakon Research International, 1984c; Donner et al., 1983). In vivo, MBT did not induce dominant lethal mutations in male rats exposed to dietary doses up to 1,300 mg/kg-day for 13 weeks prior to mating (Springborn Laboratories, 1990a, 1989a).

Chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) were statistically significantly increased by 8−37% in CHO cells exposed to MBT with metabolic activation (NTP, 1988). However, in Chinese hamster lung cells, evidence for CAs following metabolic activation was inconclusive (Matsuoka et al., 2005). Neither CAs nor SCEs were induced in either cell type without metabolic activation (Matsuoka et al., 2005; NTP, 1988). Micronuclei (MN) were not induced in human A549 lung carcinoma cells or MGC-803 gastric carcinoma epithelial cells without metabolic activation; cells were not tested with metabolic activation (Ye et al., 2014). In vivo, neither CAs nor MN were induced in mouse bone marrow following a single intraperitoneal (i.p.) injections of zinc MBT at doses up to 96 mg/kg or MBT at 300 mg/kg, respectively (Mohanan et al., 2000; Pharmakon Research International, 1984d, e).

MBT did not induce deoxyribonucleic acid (DNA) damage in *S. typhimurium* strain TA1535/pSK1002 (Ye et al., 2014). No evidence of DNA binding was observed in various rat tissues following a single gavage dose of [2-^{14}C]-labelled MBT (Brewster et al., 1989; Monsanto, 1989a).

MBT exposure at concentrations of 10.5−63 µg/mL did not induce morphological transformation of Balb/3T3 cells in vitro; cytotoxicity (survival <50% of controls) was observed at doses ≥31.3 µg/mL (Goodyear Tire & Rubber Company, 1985).

Acute Toxicity

Industrial Bio-Test Laboratories[6] (IBT Labs, 1977c) exposed single female albino rats to doses of 0, 30, 100, 300, 1,000, 3,000, or 10,000 mg/kg MBT (purity not reported) in corn oil. The only death during the 14-day observation period was the rat exposed to 10,000 mg/kg. There were no clinical signs or gross necropsy findings at sublethal doses (IBT Labs, 1977c). Younger Laboratories (1975a, 1975b) estimated oral median lethal dose (LD_{50}) values of 3,800 mg/kg (95% CI = 3,530−4,100 mg/kg) or 2,830 mg/kg (95% CI = 2,690−2,970 mg/kg) in S-D albino rats exposed to MBT powder or Thiotax MBT (respectively) and observed for 14 days. The oral rat LD_{50} for zinc MBT in corn oil was 7,500 mg zinc MBT/kg (95% CI = 6,820−8,250 mg zinc MBT/kg) under the same conditions (Younger Laboratories, 1975a). Arthur D Little (1977) reported an oral LD_{50} of 5,000 mg/kg in Swiss albino CaF1 mice exposed to MBT by gavage and observed for 7 days. Guess and O'Leary (1969) reported an oral LD_{50} of 2,000 mg/kg (95% CI = 1,798−2,225 mg/kg) in male white mice observed for 72 hours after dosing. Dow Chemical Co (1961) observed no deaths when two rats received a single oral dose of 3,980 mg/kg MBT (Captax) in corn oil; autopsy findings indicated liver and kidney injury.

IBT Labs (1977a) exposed Charles River rats by whole-body inhalation to heated dust of MBT (1,270 mg/m^3) for 4 hours and observed them for 14 days to assess acute lethality. None of the 10 rats (five/sex) died. Ptosis (eyelid drooping) and hypoactivity lasting about an hour were observed after the third hour of exposure; there were no effects on body weight or gross necropsy findings. When three rats (strain and sex not reported) were exposed via inhalation to a saturated atmosphere of MBT (concentration estimated by authors as 104 ppm) for 7 hours, there were no deaths or clinical signs, but liver and kidney damage were seen at autopsy (Dow Chemical Co, 1961).

Other Routes

The acute lethality of dermal exposure to MBT was assessed in individual male albino rabbits exposed to doses of 300, 1,000, or 3,000 mg/kg applied as an aqueous slurry to abraded skin; none of the three rabbits died (IBT Labs, 1977b). No deaths were observed when New Zealand rabbits were exposed to dermal applications of MBT, Thiotax MBT, or zinc MBT in corn oil at doses up to 7,940 mg/kg (Younger Laboratories, 1975a, b, 1974).

IBT Labs (1977b) reported that at MBT doses ≥1,000 mg/kg, erythema, mild edema, and slight desquamation of the skin at the application site were noted. Dermal hyperemia and exfoliation were noted in rat skin after 3−10 dermal applications of undiluted MBT or MBT in 10% Dowanol DPM (Dipropylene glycol methyl ether) (Dow Chemical Co, 1961). Younger Laboratories (1975a, 1975b, 1974) reported that MBT, Thiotax MBT, and zinc MBT were not

[6]A total of 618 of 867 nonacute toxicity studies conducted by Industrial Bio-Test Laboratories (including subacute, subchronic-duration, carcinogenicity, reproductive toxicity, genotoxicity, and neurotoxicity studies) were found to be invalid during a post hoc audit program conducted by U.S. EPA and the Canadian Health and Welfare Department (OECD, 2007). Discrepancies and deficiencies were also identified in acute studies, but the focus of the investigation was on repeated exposure studies that formed the basis of regulatory decisions. The laboratory closed in 1978. OECD (2007) outlined specific criteria for using data generated by Industrial Bio-Test Laboratories, and recommended rejecting a study when either a regulatory or internal audit revealed problems impacting the reliability of the findings, or when the findings of unaudited studies are inconsistent with data collected later by reputable laboratories. OECD (2007) recommended that studies that have not been audited should be used with caution and only as weak evidence if supported by later data from reputable laboratories. No information was available on internal or external auditing of this study.

irritating to the skin of rabbits when applied as a finely ground sample moistened with water at a dose of 500 mg for 24 hours.

The LD_{50} for MBT administered intraperitoneally to male white mice (observed for 72 hours) was 437 mg/kg (95% CI = 415−461 mg/kg) (Guess and O'Leary, 1969). Signs of toxicity in animals exposed to doses >335 mg/kg included marked peripheral vasodilation, salivation, and prolonged clonic and tonic seizures, suggesting central nervous system (CNS) stimulation. Pretreatment with pentobarbital (25 mg/kg, i.p.) blocked the convulsive attacks and salivation induced by high doses of MBT (550 mg/kg). When male white mice were given repeated daily i.p. injections of one-fourth and one-eighth the LD_{50} (~110 and 55 mg/kg-day) for 1 week, there were no clinical signs of toxicity or effects on body weight or gross necropsy findings (Guess and O'Leary, 1969). However, the authors reported severe histopathological liver damage in the form of extensive necrosis and disruption of anatomic organization, cloudy swelling, accumulation of cytoplasmic granules, fatty infiltration and degeneration, rupture of cell walls, and profound changes in nuclei in the high-dose mice (histopathology was not examined in low-dose mice). Diffuse, cloudy swelling of the renal tubules was also noted in exposed mice (Guess and O'Leary, 1969). Functional damage to the liver following MBT administration (110 mg/kg, i.p.) was confirmed by prolongation of sleeping time in a hexobarbital narcosis study.

In a dose range-finding study for an in vivo micronucleus assay, groups of four CD-1 mice (two/sex) were given i.p. injections of MBT at doses of 16.6, 50, 166.6, 500, or 1,666.6 mg/kg and observed for 48 hours (Pharmakon Research International, 1984d, e). All mice exposed to 1,666.6 mg/kg died, as did both females dosed with 500 mg/kg. Clinical signs were seen in the males exposed to 500 mg/kg, including decreased muscle tone and activity as well as ptosis. Some animals exposed to 50 and 166.6 mg/kg were noted to have decreased muscle tone; no signs of toxicity were seen at 16.6 mg/kg. The study authors noted the following clinical signs after a single injection of 300 mg/kg MBT: prostration, hypoactivity, hyperpnea, ptosis, tremors, and loss of righting in some animals; no deaths occurred at this dose (Pharmakon Research International, 1984d, e).

In a developmental toxicity test, MBT in dimethylsulfoxide (DMSO) was administered subcutaneously, on GDs 6−14 or 6−15 (AKR mice only), to pregnant BL6, C3H, and AKR mice at 464 mg/kg and to C3H mice at 300 mg/kg (Bionetics, 1968). Dams were allowed to litter, and offspring were evaluated at birth and PND 8, then sacrificed. In high-dose C3H mice, statistically significantly decreased fetal weight and crown-rump length (compared to controls) were observed; these effects were not seen in AKR or BL6 mice. Increased incidences of abnormal fetuses were seen in the BL6 and C3H strains exposed to 464 mg/kg, although a repeat experiment with BL6 mice failed to confirm these findings. Group sizes were small in this study (6−13 litters), limiting the reliability of the information. There was an increase in maternal liver weight (relative to body weight) in BL6 (both experiments), C3H (300 mg/kg), and AKR mice administered 200 mg/kg MBT intraperitoneally to rats on GDs 1−15 Hardin et al. (1981). The study authors reported no treatment-related histopathological effects in maternal tissues (brain, heart, lungs, liver, spleen, kidneys, adrenals, and ovaries) and no maternal toxicity (evaluated as reduced body-weight gain or statistically significant changes in two or more organ weights). No fetal toxicity (reduced pre or postimplantation survival or fetal body weight or length) nor teratogenesis (grossly visible external or internal malformations) was observed. MBT (200 mg/kg in sunflower oil, purity not reported) was injected into the stomach of female albino

rats (11−25/group) either (1) before the beginning of pregnancy on the first and third days of estrus or (2) on GDs 4 and 11 (Aleksandrov, 1982). The rats were sacrificed on GD 19. Administration of MBT before pregnancy resulted in increased duration of the estrous cycle (5.7 ± 0.3 days) compared to controls (4.4 ± 0.3 days), decreased speed to the onset of conception (1.6 ± 0.2 cycles) compared to controls (1.0 cycles), and increased postimplantation embryonic mortality ("index of mutagenicity"). Administration before or during pregnancy resulted in decreased number of corpora lutea and living fetuses and increased total percent embryonic mortality. A 23% decrease in fetal weight was also reported.

Injection of MBT or zinc MBT into 3-day-old chick embryos did not affect viability or malformations in one study (Korhonen et al., 1982). A subsequent study (Korhonen et al., 1983) reported that MBT (dose not specified) induced a 20% incidence of malformed embryos.

Metabolism/Toxicokinetic Studies

A single study provides most of the available information on toxicokinetics of MBT. El Dareer et al. (1989) examined the disposition of MBT administered by oral, intravenous (i.v.), and dermal exposure routes in rats and dermal exposure in guinea pigs. F344 rats of both sexes were dosed daily by gavage for 14 days with unlabeled MBT at 0.509 mg/kg-day prior to a single gavage dose of 0.503 mg/kg of [^{14}C]-MBT. In i.v. exposure experiments, male and female rats were given single i.v. doses of 0.602 mg/kg [^{14}C]-MBT. In dermal exposure experiments, male and female rats and female Hartley guinea pigs were topically exposed to [^{14}C]-MBT at an approximate dose of 36.1 µg/animal; the exposed area was occluded and remained unwashed for 96 hours.

After oral exposure, absorption was rapid and essentially complete in rats. Radioactivity was detected in the blood and plasma at the first time point measured (8 hours postdosing), and 90.7−101% of the administered radioactivity was excreted in the urine within 96 hours (El Dareer et al., 1989). MBT was widely distributed through the body; in rats sacrificed 8 hours after dosing, the highest levels of radioactivity were measured in the kidneys, thyroid, and liver. The study authors calculated half-lives for plasma and blood (see Table 5). The half-lives of the alpha (distribution) phase were similar for plasma and whole blood, while the beta (elimination) phases were much longer for whole blood than for plasma. To explore whether binding to erythrocytes explained the longer elimination phase, the study authors fractionated erythrocytes from exposed rats and showed that the radioactivity was primarily associated with erythrocyte cell membranes. El Dareer et al. (1989) suggested that this association might result from disulfide formation between MBT and sulfhydryl groups on the membrane, and that such binding might persist until erythrocyte turnover. The long elimination phase for plasma was postulated to result from binding to plasma proteins or erythrocyte lysis.

Table 5. Half-Lives (h) for Alpha (distribution) and Beta (elimination) Phases in Rats Exposed to ^{14}C-MBT by Gavage[a]

	Whole Blood		Plasma	
	Alpha	**Beta**	**Alpha**	**Beta**
Males	4.7	6,000	4.12	312
Females	8.56	5,180	3.73	79.6

[a]El Dareer et al. (1989).

MBT = 2-mercaptobenzothiazole.

Experiments in rats intravenously dosed showed similar plasma and whole blood elimination phases, similar tissue distribution, and urinary excretion (El Dareer et al., 1989). In the dermal exposure experiments, guinea pigs absorbed a greater percentage of the MBT than rats (33.4 vs. 16.1−17.5%), but the urinary excretion levels were similar (91% of the absorbed dose was excreted in urine in male rats, 94% in female rats, and 98% in female guinea pigs).

Metabolites of MBT excreted in rat urine within 8 hours after gavage dosing were analyzed by high performance liquid chromatography and spectrophotometry (El Dareer et al., 1989). Only two metabolites were identified, and unchanged MBT was not detected in urine. The major metabolite (relative quantities were not reported) was determined to be a thioglucuronide of MBT, and the second was a sulfonic acid derivative of MBT (El Dareer et al., 1989). In a study of guinea pigs exposed dermally to MBT, glucuronide and sulfate derivatives of MBT were reportedly detected in urine [Nagamatsu et al. (1979) as cited in El Dareer et al. (1989)].

Immunotoxicity

Ahuja et al. (2009) tested the sensitizing potential of MBT in a biphasic local lymph node assay using dermal and oral experimental protocols. Female Balb/c mice were sensitized via 3 days of dermal exposure to concentrations of 3, 10, or 30% or three daily gavage doses of 1, 10, or 100 mg/kg. The animals in both groups were subsequently challenged with auricular applications of 3, 10, or 30% concentrations of MBT on Days 15−17. At the end of the challenge, ear thickness was measured, auricular lymph nodes were weighed, and lymph node cells were counted and assayed by flow cytometry for lymphocyte subtype counts. In the dermal study, statistically significant increases in total lymphocyte cell count and lymph node weights, and a statistically significant decrease in CD8+ cells, were observed at the low and mid MBT exposure concentrations, but not at the highest concentration. In the oral study, a statistically significant increase in total cell count was observed at 1 mg/kg (but not at higher doses), and increased lymph node weight was observed at 10 mg/kg. The study authors concluded that MBT is a mild to moderate allergen with sensitizing potential after oral and dermal exposure (Ahuja et al., 2009).

Neurotoxicity

Indications of neurotoxicity have been reported in several studies; however, consistent evidence for neurotoxic mechanisms or effects is not available. As discussed in the "Other Routes" section, acute i.p. administration of MBT (>335 mg/kg) induced CNS stimulation, which was blocked by pretreatment with pentobarbital (Guess and O'Leary, 1969). Other studies described in the "Oral Exposures" section of this document report lethargy following dosing, salivation, irritable behavior increasing with dose, and resistance to gavage. Additionally, a repeat-dose neurotoxicity study in S-D rats did not find evidence of altered motor activity or FOB parameters following dietary MBT exposure for 13 weeks (up to 1,920.4 mg/kg-day) (Bio-Research Laboratories LTD, 1990).

Bio-Research Laboratories LTD (1989) conducted an acute oral neurotoxicity study in groups of 12 male and 12 female S-D rats. Single doses of 0, 500, 1,250, or 2,750 mg/kg MBT (purity not reported) in corn oil were administered by gavage. Before dosing and during the 14-day observation period, the animals were examined in a FOB (1, 6, and 24 hours after dosing and on Days 7 and 14). Motor activity was evaluated before exposure and at 12 hours after dosing. All animals were necropsied at the end of the observation period. One female rat dosed with 2,750 mg/kg MBT died 12 hours after exposure. Males in the high-dose group and all dosed females exhibited muzzle staining and increased incidences of salivation. Decreased vocalization was noted in males exposed to ≥1,250 mg/kg when observed 1 and 6 hours after dosing, and increased urinary staining was noted in high-dose females 24 hours after dosing. Motor activity was statistically significantly reduced in females at doses ≥1,260 mg/kg and in high-dose males; grip strength and hindlimb splay were not affected by exposure. There were no notable gross necropsy findings. The study authors suggested that the observed effects may be related to nonspecific acute toxicity rather than indicating neurotoxicity.

Intraperitoneal injection of 300 mg/kg MBT in mice (strain and sex not specified) resulted in reduced noradrenaline (60% less than controls) 1 and 2 hours after dosing, as well as increased dopamine (24% higher) when assayed 2 hours after dosing (Johnson et al., 1970). The animals were observed to be "extremely depressed," with markedly reduced spontaneous motor activity after dosing. Marked ptosis occurred after 2 hours. By 4 hours after dosing, the animals behaved as the controls (Johnson et al., 1970). The study authors also reported that MBT

noncompetitively inhibited bovine dopamine β-hydroxylase in vitro and postulated that chelation of copper ion (which is critical to the enzyme activity) was the mechanism by which the catecholamine levels were altered in vivo.

DERIVATION OF PROVISIONAL VALUES

Tables 6 and 7 present summaries of noncancer and cancer references values, respectively.

Table 6. Summary of Noncancer Reference Values for MBT (CASRN 149-30-4)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/female	Increased relative liver weight	4×10^{-2}	$BMDL_{10}$	3.56	100	NTP (1988)
Chronic p-RfD (mg/kg-d)	Rat/female	Increased relative liver weight	4×10^{-3}	$BMDL_{10}$	3.56	1,000	NTP (1988)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole; NDr = not determined; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; UF_C = composite uncertainty factor.

Table 7. Summary of Cancer Reference Values for MBT (CASRN 149-30-4)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	Rat/female	Combined tumors	1.1×10^{-2}	NTP (1988)
p-IUR $(mg/m^3)^{-1}$	NDr			

MBT = 2-mercaptobenzothiazole; NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

DERIVATION OF ORAL REFERENCE DOSES

Studies of MBT that are potentially relevant to the derivation of provisional reference dose (p-RfD) values include a short-term-duration range-finding study in rats exposed via the diet (Monsanto, 1989b), short-term-duration range-finding studies in rats and mice exposed by gavage (NTP, 1988), a subchronic neurotoxicity study in rats exposed via the diet (Bio-Research Laboratories LTD, 1990), subchronic and chronic toxicity studies in rats and mice exposed by gavage (NTP, 1988), a two-generation reproductive toxicity study in rats exposed via the diet (Springborn Laboratories, 1990b), and developmental toxicity studies in rats and rabbits exposed

by gavage (Springborn Laboratories, 1989b, c, d, e). Subchronic and chronic p-RfDs were derived based on the available studies.

Derivation of a Subchronic Provisional Reference Dose (p-RfD)

The subchronic-duration study of adult rats exposed to MBT by gavage is considered the principal study for derivation of the subchronic p-RfD (NTP, 1988). The critical effect from this study is increased relative liver weight in female rats.

The subchronic toxicity studies conducted by NTP (1988) report administration of MBT by gavage to F344/N rats and $B6C3F_1$ mice (10/sex/dose) for 13 weeks. These studies are included in a peer-reviewed technical report conducted according to Good Laboratory Practice (GLP) standards with adequate numbers of dose groups, appropriate dose spacing, sufficient group sizes, and quantitation of results to describe dose-response relationships for the critical effects. However, uncertainty remains due to the lack of comprehensive endpoint evaluation; hematology, clinical chemistry, urinalysis, and organ-weight measurements for organs other than liver were not performed or reported. Short-term exposure studies in rats and mice were not selected as principal studies because of the brief exposure durations (16−28 days). The subchronic exposure neurotoxicity study by Bio-Research Laboratories LTD (1990) reported reduced body weight that occurred in the context of reduced food intake potentially resulting from poor palatability of the test substance in the diet and was thus not considered as the principal study. Among the remaining studies, the most sensitive effects were increased absolute and relative liver weight in male rats and female rats and mice (NTP, 1988) (see Table 8). Reproductive and developmental studies were not selected as principal studies because effects resulted from higher doses than the dose producing organ-weight changes in adult rats reported by NTP (1988). In the two-generation reproductive study in rats (Springborn Laboratories, 1990b), a LOAEL of 172.1 mg/kg-day was identified based on increased incidence of basophilic tubules in male F0 rats and a >10% increase in relative liver weight in male F1 rats, consistent with effects observed by NTP (1988). In the developmental toxicity studies in rats, a developmental LOAEL of 300 mg/kg-day was identified for an increase in postimplantation loss (Springborn Laboratories, 1989e). In the developmental toxicity range-finding study in rabbits, a developmental LOAEL (lowest dose tested) of 150 mg/kg-day was identified due to >10% decreased fetal weight; however, the follow-up developmental study did not replicate this fetal toxicity with larger sample sizes and a NOAEL of 300 mg/kg-day was identified for maternal and developmental toxicity in rabbits (Springborn Laboratories, 1989b, d).

The subchronic-duration studies conducted by NTP (1988) reported biologically significant (i.e., >10% compared to control) increases in absolute and relative liver weight at all treatment doses in rats and biologically significant increases in relative liver weight at all doses and in absolute liver weight at doses ≥536 mg/kg-day in mice (see Table 8). Few studies measured organ weight following MBT exposure; however, the three other studies that examined liver weight also reported increased relative liver weight in exposed rats and rabbits (Springborn Laboratories, 1990b; Monsanto, 1989b; Springborn Laboratories, 1989d). The reproductive study in rats by Springborn Laboratories (1990b) reported increased absolute and relative liver weights in F0 and F1 male and female parents. These organ-weight changes were accompanied by histopathological findings of increased incidences of hepatocellular hypertrophy. Liver damage and weight changes have also been observed following exposure to MBT via other routes (i.e., subcutaneous, i.p.) and exposure durations (i.e., acute) (Guess and O'Leary, 1969; Bionetics, 1968; Dow Chemical Co, 1961). The LOAEL and lowest dose tested of

134 mg/kg-day for increased absolute and/or relative liver weight in male and female rats and LOAEL of 67 mg/kg-day for increased relative liver weight in female mice are lower than all other LOAEL values in the database and were selected for benchmark dose (BMD) modeling (see Table 9).

Table 8. Selected Non-neoplastic Endpoints in F344 Rats and B6C3F$_1$ Mice Exposed by Gavage to MBT for 13 Weeks[a,b]

Male Rats					
Average daily dose (mg/kg-d)	**0**	**134**	**268**	**536**	**1,071**
Number of animals	10	10	9	10	8
Absolute liver weight (mg)	13,593 ± 2,121	15,661 ± 793 (+15%)	15,861 ± 1,712 (+17%)	18,742 ± 2,631** (+38%)	16,759 ± 2,660** (+23%)
Relative liver weight (mg/g)	38.4 ± 6.07	43.9 ± 1.87* (+14%)	47.2 ± 2.79** (+23%)	54.8 ± 5.08** (+43%)	51.3 ± 5.42** (+34%)
Female Rats					
Average daily dose (mg/kg-d)	**0**	**134**	**268**	**536**	**1,071**
Number of animals	10	9	10	8	10
Absolute liver weight (mg)	6,606 ± 795	7,818 ± 814** (+18%)	8,027 ± 688** (+22%)	7,988 ± 591** (+21%)	8,413 ± 652** (+27%)
Relative liver weight (mg/g)	31.8 ± 3.28	39.3 ± 3.53** (+24%)	39.9 ± 2.99** (+25%)	41.8 ± 2.81** (+31%)	43.2 ± 2.61** (+36%)

Female Mice						
Average daily dose (mg/kg-d)	**0**	**67**	**134**	**268**	**536**	**1,071**
Number of animals	10	10	10	10	8	3[c]
Absolute liver weight (mg)	1,129 ± 242	1,237 ± 123 (+9.6%)	1,238 ± 113 (+9.7%)	1,232 ± 124 (+9.1%)	1,281 ± 126 (+13%)	1,383 ± 96 (+22%)
Relative liver weight (mg/g)	42.9 ± 7.71	48.6 ± 5.03 (+13%)	47.9 ± 3.61 (+12%)	47.8 ± 3.74 (+11%)	49.2 ± 4.70 (+15%)	54.7 ± 3.45** (+28%)

[a]NTP (1988).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Statistically significant mortality (7/10).
*Statistically significantly different from control at $p \leq 0.05$, as reported by the study authors (Dunnett's test).
**Statistically significantly different from control at $p \leq 0.01$, as reported by the study authors (Dunnett's test).

MBT = 2-mercaptobenzothiazole.

Potential points of departure (PODs) from NTP (1988) were modeled using the EPA's Benchmark Dose Software (BMDS, Version 2.6) (see Table 8). The results are summarized in Table 9. A benchmark response (BMR) of 10% relative deviation (RD) from the control mean was used. Appendix C contains details of the modeling. No model fit was achieved with the

data on absolute and relative liver weight in male rats, absolute liver weight in female rats, or absolute and relative liver weight in female mice due to the nonlinearity of the dose response. Modeling of the data on relative liver weight in female rats yielded a 10% benchmark dose lower confidence limit ($BMDL_{10}$) estimate of 14.82 mg/kg-day.

Table 9. Potential Subchronic PODs in F344 Rats and B6C3F$_1$ Mice Exposed by Gavage to MBT for 13 Weeks[a]

Endpoint	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Animal POD[b] (mg/kg-d)	POD (HED)[c] (mg/kg-d)
Male Rats				
Absolute liver weight	ND	134	LOAEL = 134	LOAEL (HED) = 32.2
Relative liver weight	ND	134	LOAEL = 134	LOAEL (HED) = 32.2
Female Rats				
Absolute liver weight	ND	134	LOAEL = 134	LOAEL (HED) = 32.2
Relative liver weight[d]	**ND**	**134**	**$BMDL_{10}$ = 14.8**	**$BMDL_{10}$ (HED) = 3.56**
Female Mice				
Absolute liver weight	268	536	NOAEL = 268	NOAEL (HED) = 37.5
Relative liver weight	ND	67	LOAEL = 67	LOAEL (HED) = 9.4

[a]NTP (1988).
[b]BMD modeling results are described in detail in Appendix C.
[c]POD (HED) = Animal POD (mg/kg-day) × DAF of 0.24 for rats or 0.14 for mice (U.S. EPA, 2011b).
[d]Chosen as the critical effect for derivation of the subchronic p-RfD.

BMD = benchmark dose; $BMDL_{10}$ = 10% benchmark dose lower confidence limit; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; MBT = 2-mercaptobenzothiazole; ND = no data; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose.

In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches may include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In lieu of chemical-specific models or data to inform the derivation of human equivalent oral exposures, EPA endorses $BW^{3/4}$ as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving an RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite, but not for portal-of-entry effects.

A validated human physiologically based pharmacokinetic (PBPK) model for MBT is not available for use in extrapolating doses from animals to humans. In addition, liver-weight changes are not a portal-of-entry effect. Therefore, scaling by $BW^{3/4}$ is relevant for deriving HED for this effect.

Following U.S. EPA (2011b) guidance, all potential PODs in rats and mice are converted to a HED through the application of a DAF[7] derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where:

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a reference BW_a of 0.25 kg for rats and 0.025 kg for mice, and a reference BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAFs are 0.24 and 0.14 for rats and mice, respectively. Each POD candidate is multiplied by the appropriate species-specific DAF to obtain POD (HED) (see Table 9).

Absolute and relative liver-weight changes in male and female rats were biologically significant at the lowest dose tested (i.e., LOAEL = 134 mg/kg-day); thus, no NOAEL was identified in these animals. Relative liver-weight changes in female mice were also observed at the lowest dose tested (LOAEL = 67 mg/kg-day); however, increases in absolute liver weight in these animals were not biologically significant until higher dose levels (LOAEL = 536 mg/kg-day). This multiple dose level difference in the LOAEL between absolute and relative liver-weight changes in female mice lends uncertainty to the biological significance of the relative-weight change in female mice. The lowest POD (HED) following subchronic exposure to MBT is increased relative liver weight in female rats ($BMDL_{10}$ [HED] = 3.56 mg/kg-day), which was the only potential POD that resulted in a suitable BMDS model fit. This POD (HED) allows for a better representation of the full range of the dose-response for liver-weight changes in rats compared to the other potential PODs. Therefore, it is concluded that the $BMDL_{10}$ (HED) for relative liver weight in female rats is protective of other effects observed following MBT exposure. Based on the consistency and coherence in effects on the liver across species and sexes and biological significance of the changes, **the $BMDL_{10}$ (HED) for relative liver-weight increases in female rats (3.56 mg/kg-day) is selected as the POD for derivation of the subchronic p-RfD.**

Subchronic p-RfD	=	$BMDL_{10}$ (HED) ÷ UF_C
	=	3.56 mg/kg-day ÷ 100
	=	**4×10^{-2} mg/kg-day**

The composite uncertainty factor (UF_C) for the subchronic p-RfD for MBT is 100, as summarized in Table 10.

[7]As described in detail in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), rate-related processes scale across species in a manner related to both the direct ($BW^{1/1}$) and allometric scaling ($BW^{3/4}$) aspects such that $BW^{3/4} \div BW^{1/1} = BW^{-1/4}$, converted to a $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Table 10. Uncertainty Factors for the Subchronic p-RfD for MBT

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following MBT exposure. The toxicokinetic uncertainty has been accounted for by calculating a HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of MBT in humans.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database. The available subchronic- and chronic-duration toxicity studies for MBT are not comprehensive. However, the database includes a two-generation reproductive toxicity study in rats (Springborn Laboratories, 1990b), developmental toxicity studies in rats and rabbits (Springborn Laboratories, 1989d, e), and subchronic-duration and acute neurotoxicity studies in rats (Bio-Research Laboratories LTD, 1990, 1989), all via the oral route.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because the POD is from a subchronic-duration study.
UF_C	100	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; DAF = dosimetric adjustment factor; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole; POD = point of departure.

Confidence in the subchronic p-RfD for MBT is medium as explained in Table 11.

Table 11. Confidence Descriptors for the Subchronic p-RfD for MBT

Confidence Categories	Designation	Discussion
Confidence in principal study	M	Confidence in the principal study is medium. The study by NTP (1988) is a well-conducted, peer-reviewed, GLP-compliant study of oral exposure to MBT; however, hematology, clinical chemistry, urinalysis, and organ-weight measurements for organs other than liver were not assessed.
Confidence in database	M	Confidence in the subchronic database is medium. The database includes subchronic-duration studies in rats and mice, a two-generation reproductive toxicity study in rats, developmental toxicity studies in rats and rabbits, and subchronic and acute neurotoxicity studies in rats. However, the available subchronic-duration studies were not comprehensive and lacked hematology, clinical chemistry, urinalysis, and organ-weight measurements for organs other than liver.
Confidence in subchronic p-RfD[a]	M	The overall confidence in the subchronic p-RfD for MBT is medium.

[a]The overall confidence cannot be greater than the lowest entry in a table.

GLP = Good Laboratory Practice; M = medium; MBT = 2-mercaptobenzothiazole; p-RfD = provisional reference dose.

Derivation of a Chronic Provisional Reference Dose (p-RfD)

After evaluation of the available oral exposure studies, it was determined that the same critical effect and principal study as the subchronic p-RfD was adequate for deriving a chronic p-RfD. The chronic-duration studies of MBT administration conducted by NTP (1988) cannot be used to derive noncancer toxicity values because effect levels could not be determined for these studies due to the occurrence of tumors at the tested doses (see study summary for additional details). The chronic-duration study by NTP (1988) did report increased incidence of forestomach lesions in male and female rats at 268 mg/kg-day. Males had increased ulcers, inflammation, epithelial hyperplasia, and hyperkeratosis, and females had increased ulcers. However, no changes in the forestomach were observed in the subchronic-duration study, and liver weight was not evaluated in the chronic-duration study. Therefore, there is uncertainty whether a POD based on the lesions in the forestomach would be protective against the potentially more sensitive changes in the liver. In addition, a greater body of evidence supports the changes in the liver compared to the forestomach as discussed in the "Derivation of a Subchronic Provisional Reference Dose" section. Thus, it is concluded that the $BMDL_{10}$ (HED) for relative liver weight in female rats is protective of other effects observed following MBT exposure. Based on the consistency and coherence in effects on the liver across species and sexes and biological significance of the changes, **the $BMDL_{10}$ (HED) for relative liver-weight increases in female rats (3.56 mg/kg-day) is selected as the POD for derivation of the chronic p-RfD.**

The chronic p-RfD was derived as follows:

Chronic p-RfD	=	$BMDL_{10}$ (HED) ÷ UF_C
	=	3.56 mg/kg-day ÷ 1,000
	=	**4×10^{-3} mg/kg-day**

Table 12 summarizes the UF_C for the chronic p-RfD for MBT.

Table 12. Uncertainty Factors for the Chronic p-RfD for MBT

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following MBT exposure. The toxicokinetic uncertainty has been accounted for by calculating a HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of MBT in humans.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database. The available subchronic- and chronic-duration toxicity studies for MBT are not comprehensive. However, the database includes a two-generation reproductive toxicity study in rats (Springborn Laboratories, 1990b), developmental toxicity studies in rats and rabbits (Springborn Laboratories, 1989d, e), and subchronic-duration and acute neurotoxicity studies in rats (Bio-Research Laboratories LTD, 1990, 1989), all via the oral route. However, only the NTP (1988) studies were peer reviewed; the remaining studies were unpublished and not peer reviewed.
UF_L	1	A UF_L of 1 is applied because POD is a BMDL.
UF_S	10	A UF_S of 10 is applied to account for the extrapolation from less than chronic exposure.
UF_C	1,000	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; DAF = dosimetric adjustment factor; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole; POD = point of departure.

Confidence in the chronic p-RfD for MBT is medium as explained in Table 13.

Table 13. Confidence Descriptors for the Chronic p-RfD for MBT

Confidence Categories	Designation	Discussion
Confidence in principal study	M	Confidence in the principal study is medium. The study by NTP (1988) is a well-conducted, peer-reviewed, GLP-compliant study of oral exposure to MBT; however, hematology, clinical chemistry, urinalysis, and organ-weight measurements for organs other than liver were not assessed.
Confidence in database	M	Confidence in the chronic database is medium. The database includes chronic-duration studies in rats and mice, a two-generation reproductive toxicity study in rats, developmental toxicity studies in rats and rabbits, subchronic-duration studies in rats and mice, and acute neurotoxicity studies in rats. However, the available chronic-duration studies were not comprehensive and lacked hematology, clinical chemistry, urinalysis, and organ-weight measurements.
Confidence in chronic p-RfD[a]	M	The overall confidence in the chronic p-RfD for MBT is medium.

[a]The overall confidence cannot be greater than the lowest entry in a table.

GLP = Good Laboratory Practice; M = medium; MBT = 2-mercaptobenzothiazole; p-RfD = provisional reference dose.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies identifying non-neoplastic effects of MBT in humans or animals exposed by inhalation have been identified in the literature reviewed, precluding derivation of RfCs.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Tables 14 and 15 provide the cancer weight-of-evidence (WOE) descriptors for oral and inhalation exposure to MBT, respectively.

Table 14. Cancer WOE Descriptor for Oral Exposure to MBT

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	The available data do not support this.
"Likely to Be Carcinogenic to Humans"	**Selected**	**Oral**	**NTP (1988) conducted carcinogenicity studies in rats and mice and concluded that there was *some evidence of carcinogenicity* in male rats based on statistically significant increases in the incidences of mononuclear cell leukemia, pancreatic acinar cell adenomas, adrenal gland pheochromocytomas, and preputial gland adenomas or carcinomas. NTP indicated that there was *some evidence of carcinogenicity* in female rats based on statistically significant increases in the incidences of adrenal gland pheochromocytomas and pituitary gland adenomas. For mice, NTP concluded that there was *no evidence of carcinogenicity* in male mice and *equivocal evidence of carcinogenicity* in female mice based on increased incidences of hepatocellular adenomas or carcinomas.**
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	The available data do not support this, as multiple tumor types were observed in 2 sexes and species of animals (male and female rats and female mice) (NTP, 1988).
"Inadequate Information to Assess Carcinogenic Potential"	NS	NA	The available data do not support this. Data are available demonstrating increased tumor incidence in animals (NTP, 1988).
"Not Likely to Be Carcinogenic to Humans"	NS	NA	The available data do not support this.

MBT = 2-mercaptobenzothiazole; NA = not applicable, NS = not selected; NTP = National Toxicology Program; WOE = weight of evidence.

Table 15. Cancer Weight-of-Evidence Descriptor for Inhalation of MBT

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	The available data do not support this. The available epidemiological studies suffer from a number of limitations that preclude robust conclusions regarding carcinogenic potential (see text for detail). There are no carcinogenicity studies in animals exposed by inhalation.
"Likely to Be Carcinogenic to Humans"	NS	NA	The available data do not support this. The available epidemiological studies suffer from a number of limitations that preclude robust conclusions regarding carcinogenic potential (see text for detail). There are no carcinogenicity studies in animals exposed by inhalation.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	The available data do not support this. The available epidemiological studies suffer from a number of limitations that preclude robust conclusions regarding carcinogenic potential (see text for detail). There are no carcinogenicity studies in animals exposed by inhalation.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Inhalation**	**Several epidemiological studies investigated cancer morbidity and mortality in 2 cohorts of rubber factory workers with MBT inhalation exposure (Sorahan, 2009, 2008; Sorahan et al., 2000; Collins et al., 1999; Sorahan and Pope, 1993; Strauss et al., 1993). These studies suggested potential associations between MBT and bladder cancer, colon cancer, and multiple myeloma. However, the studies of these cohorts suffer from a number of limitations that preclude robust conclusions regarding carcinogenic potential (see text for detail). There are no carcinogenicity studies in animals exposed by inhalation.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	The available data do not support this.

MBT = 2-mercaptobenzothiazole; NA = not applicable, NS = not selected; WOE = weight of evidence.

In accordance with U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment,* oral exposure to MBT is *"Likely to be Carcinogenic to Humans."* There are no human data on the potential carcinogenicity of MBT by oral exposure. The carcinogenicity of MBT has been tested in three chronic-duration studies of rats and mice exposed orally (NTP, 1988; Innes et al., 1969). Chronic exposure (103 weeks) to MBT via gavage resulted in statistically significantly increased incidences of tumors in male and female rats and female mice, including statistically significant increases in the incidences of mononuclear cell leukemia, pituitary gland adenomas, pancreatic acinar cell adenomas, mesothelioma, adrenal gland pheochromocytomas, fibroma, neurofibroma, sarcoma, or fibrosarcomas, and preputial gland adenomas or carcinomas in male rats (NTP,

1988). Statistically significant increases in the incidences of adrenal gland pheochromocytomas and pituitary gland adenomas occurred in female rats, and increased incidences of hepatocellular adenomas or carcinomas occurred in female mice (NTP, 1988). In the rat study, NTP (1988) concluded that there was some evidence of carcinogenicity in males based on statistically significant increases in the incidences of mononuclear cell leukemia, pancreatic acinar cell adenomas, adrenal gland pheochromocytomas, and preputial gland adenomas or carcinomas. NTP (1988) also concluded that there was some evidence of carcinogenicity in females based on statistically significant increases in the incidences of adrenal gland pheochromocytomas and pituitary gland adenomas. In the mouse study, NTP concluded that there was no evidence of carcinogenicity in male mice and equivocal evidence of carcinogenicity in female mice based on increased incidences of hepatocellular adenomas or carcinomas. Increased incidence of tumors was not reported in mice exposed to MBT or zinc MBT for 18 months (Innes et al., 1969); however, quantitative results were not presented.

As stated in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), supporting data to conclude that a chemical is *"Likely to Be Carcinogenic to Humans"* includes "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans." Based on this example and the data available in male and female rats and mice, exposure to MBT is *"Likely to Be Carcinogenic to Humans."*

In accordance with U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*, the database for inhalation exposure to MBT provides *"Inadequate Information to Assess Carcinogenic Potential."* No chronic-duration animal inhalation studies of MBT were identified. Several epidemiological studies investigated cancer morbidity and mortality in two cohorts of rubber factory workers with MBT inhalation exposure (Sorahan, 2009, 2008; Sorahan et al., 2000; Collins et al., 1999; Sorahan and Pope, 1993; Strauss et al., 1993). These studies suggested potential associations between MBT and bladder cancer, colon cancer, and multiple myeloma. As discussed earlier, the studies of these cohorts suffer from a number of limitations. They include:

- The numbers of workers with likely MBT exposure in both cohorts were small (≤600 workers each), and the numbers of cancers observed were also small.
- Both worker cohorts had potential exposure to MBT, its derivatives, and other chemicals, including the known or suspected bladder carcinogens, PAB and PBN.
- MBT exposure assessments for both cohorts were based on job-exposure matrices, using limited exposure monitoring information.
- Data on tobacco use were not available for either cohort, raising the possibility of confounding.
- Follow-up studies of the same cohort and studies of the different cohorts did not provide consistent findings, possibly because the numbers of cases and the sizes of the cohorts were too small to yield stable results.

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005) define mode of action (MOA) "…as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." Examples of possible modes of carcinogenic action for any given chemical include

"mutagenicity, mitogenesis, programmed cell death, cytotoxicity with reparative cell proliferation, and immune suppression" (pp. 1−10).

The MOA for carcinogenic effects of MBT is not known. In general, available data indicate that MBT is not mutagenic. Limited in vitro data suggest that MBT may cause clastogenic effects in mammalian cells; however, findings are inconsistent between studies and cell types, and in vivo data were negative (see "Genotoxicity" section for more details). No other information suggesting potential MOA(s) for MBT carcinogenicity is available. Thus, a linear approach to derivation of provisional cancer potency values is applied as recommended by the *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of a Provisional Oral Slope Factor (p-OSF)

The 2-year carcinogenicity studies in rats and mice conducted by NTP (1988) were selected as the principal studies for the development of a p-OSF. This study was conducted in accordance with GLP principles, the results are peer reviewed, and the study meets the standards of study design and performance with respect to the number of animals used, the examination of potential toxicity endpoints, and the presentation of information.

Statistically significant increases in the incidences of mononuclear cell leukemia, pituitary gland adenomas, pancreatic acinar cell adenomas, mesothelioma, adrenal gland pheochromocytomas, fibroma, neurofibroma, sarcoma, or fibrosarcomas and preputial gland adenomas or carcinomas in male rats; significant increases in the incidences of adrenal gland pheochromocytomas and pituitary gland adenomas in female rats; and increased incidences of hepatocellular adenomas or carcinomas in female mice were reported (NTP, 1988). Several tumor types observed at statistically significantly increased incidence were not considered for deriving the p-OSF. NTP (1988) noted that a number of tumor types observed in male rats did not exhibit monotonic dose-response relationships. As shown in Table B-4, the incidences of mononuclear cell leukemia, pituitary gland adenomas, and pancreatic acinar cell adenomas in male rats suggested inverted U-shaped dose-response relationships (incidences declined from the low to the high dose, often to incidences at or below the control values). Survival of male rats was statistically significantly reduced by treatment; however, NTP (1988) indicated that the lack of a monotonic dose-response relationship was not likely to be attributable to increased mortality, because comparable numbers of male rats in the low- and high-dose groups were at risk at the end of the study. In addition, significant dose-related trends were not seen in life table or incidental tumor tests (accounting for mortality) for mononuclear cell leukemia or pituitary gland adenomas in male rats. While pancreatic acinar cell adenomas exhibited a statistically significant trend by life table test, adjusted incidence rates (4.5, 45.7, and 23% in control, low-dose, and high-dose groups) reported by NTP (1988) also did not exhibit a dose-response relationship, indicating that adjustment for mortality would not yield a monotonic dose-response relationship. Therefore, based on the lack of dose-response relationship, these tumor incidence data were not used for BMD modeling. Similarly, while low-dose female mice exhibited statistically significantly increased incidence of liver tumors, the dose-response was not monotonic and there was no significant dose-related trend by either life table or incidental tumor test (NTP, 1988); thus, this tumor type was not used. The remaining data for tumors occurring in male and female rats were selected for BMD modeling and are provided in Table 16.

Table 16. Incidences of Selected Neoplastic Lesions in F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Male Rats			
HED, mg/kg-d	**0**	**64.3**	**129**
Mesothelioma	0/50[†]	2/50 (4%)	3/50 (6%)
Adrenal gland			
Pheochromocytoma	18/50[†] (36%)	25/50* (50%)	22/49* (45%)
Pheochromocytoma or malignant pheochromocytoma	18/50[†] (36%)	27/50*[#] (54%)	24/49*[#] (49%)
Preputial gland			
Adenoma	0/50[†]	4/50* (8%)	4/50* (8%)
Adenoma or carcinoma	1/50[†] (2%)	6/50* (12%)	5/50* (10%)
Subcutaneous tissue			
Fibroma	2/50[†] (4%)	3/50 (6%)	6/50* (12%)
Fibroma, neurofibroma, sarcoma, or fibrosarcoma	3/50[†] (6%)	6/50 (12%)	7/50* (14%)
Female Rats			
HED, mg/kg-d	**0**	**32.2**	**64.3**
Pituitary gland			
Adenoma	15/49[†] (31%)	24/50 (48%)	25/50*[#] (50%)
Adenoma or adenocarcinoma	16/49[†] (33%)	24/50 (48%)	25/50*[#] (50%)
Adrenal gland, pheochromocytoma	1/50[†] (2%)	5/50 (10%)	6/50* (12%)

[a]NTP (1988).
*Statistically significantly different from concurrent control at $p < 0.05$ based on life table test performed by the study authors.
[#]Statistically significantly different from concurrent control at $p < 0.05$ based on incidental tumor test performed by the study authors.
[†]Statistically significant ($p < 0.05$) dose-related trend by life table or incidental tumor analysis or both.

HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

The incidences of the various tumor types were modeled using BMDS, and the modeling results are presented in Table 17. Based on the BMD modeling results, the calculated cancer slope factors for the various tumor types in male and female rats were calculated and also presented in Table 17. Because treatment with MBT produced multiple types of tumors in different tissues within a single study (i.e., within a single sex and species), the overall oral cancer slope factor for MBT exposure was derived based on the incidence data for combined tumors assuming that different tumor types are independent from each other. The overall tumor incidence was fit with the MS_Combo multiple tumor model (BMDS, Version 2.6; see Appendix C for details), and the estimated BMD_{10}, $BMDL_{10}$, and calculated cancer slope

factors are presented in Table 17.[8] This modeling provides an estimate of composite risk for developing any combination of tumors at any site within a single study. Modeling procedures and results are described in detail in Appendix C.

Prior to modeling, all doses were converted to HEDs using $BW^{3/4}$ scaling, as described below.

$$\text{Dose (HED)} = \text{Dose} \times (BW_a/BW_h)^{1/4}$$

where:

Dose = average daily animal dose of MBT
BW_a = reference animal body weight[9]
BW_h = 70 kg, reference human body weight (U.S. EPA, 1988)

Using a BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans, the resulting default DAF is 0.24 (U.S. EPA, 2011b, 2005).

[8]Because no software is currently available to estimate composite risk from multiple tumor types and adjust for group differences in survival, data for tumor incidence in male rats were also modeled using a Poly-3 survival-adjusted number at risk to account for decreased survival. Modeling the observed incidence and Poly-3 weighted number give a $BMDL_{10}$ (HED) = 10.8, which is higher than the $BMDL_{10}$ (HED) of 8.91 for combined tumors in female rats.

[9]Time-weighted body weight was not reported by study authors or calculable from reported study data. Default animal body weights (0.25 kg for rats and 0.025 kg for mice) were used in calculating HED values.

Table 17. Comparison of BMD Model Results for Possible Tumor Endpoints for Derivation of the p-OSF[a]

Tumor Endpoint	Model Type	Goodness-of-Fit *p*-Value[b]	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)	Potential p-OSF $(mg/kg\text{-}d)^{-1}$
Female Rats					
Adrenal gland pheochromocytoma	Multistage cancer (1st order)	0.5478	55.0	30.5	3.3×10^{-3}
Pituitary gland adenoma or adenocarcinoma	Multistage cancer (1st order)	0.4873	21.0	10.9	9.2×10^{-3}
Combined tumors	**MS_Combo**		**15.2**	**8.91**	$\mathbf{1.1 \times 10^{-2}}$
Male Rats					
Mesothelioma	Multistage cancer (1st order)	1.000	333	147	6.8×10^{-4}
Adrenal gland pheochromocytoma or malignant pheochromocytoma	Multistage cancer (1st order)	0.1991	51.1	23.0	4.3×10^{-3}
Preputial gland adenoma or carcinoma	Multistage cancer (1st order)	0.2107	118	62.5	1.6×10^{-3}
Subcutaneous tissue fibroma, neurofibroma, sarcoma, or fibrosarcoma	Multistage cancer (1st order)	0.7217	142	63.7	1.6×10^{-3}
Combined tumors	MS_Combo		24.9	15.0	6.7×10^{-3}

[a]NTP (1988).
[b]Values >0.05 meet conventional goodness-of-fit criteria.

BMD = benchmark dose; BMD_{10} = 10% benchmark dose; $BMDL_{10}$ = 10% benchmark dose lower confidence limit; HED = human equivalent dose; p-OSF = provisional oral slope factor.

Among all of the modeled tumor types, the lowest POD ($BMDL_{10}$ [HED] = 8.91 mg/kg-day) was obtained in modeling of the incidence of combined tumors in female rats. The MOA by which MBT induces tumors is not known. In the absence of definitive information, a linear approach is used to obtain the slope from the POD. The **p-OSF of $\mathbf{1.1 \times 10^{-2}}$ $\mathbf{(mg/kg\text{-}day)^{-1}}$** was derived as follows:

$$\begin{aligned} \textbf{p-OSF} &= BMR \div BMDL_{10}\ (HED) \\ &= 0.1 \div 8.91\ mg/kg\text{-}day \\ &= \mathbf{1.1 \times 10^{-2}\ (mg/kg\text{-}day)^{-1}} \end{aligned}$$

APPENDIX A. SCREENING PROVISIONAL VALUES

No screening values for 2-mercaptobenzothiazole are identified.

APPENDIX B. DATA TABLES

Table B-1. Absolute and Relative Liver Weights in F344 Rats Exposed by Gavage to MBT for 13 Weeks[a,b]

Male Rats					
Average daily dose, mg/kg-d	**0**	**134**	**268**	**536**	**1,071**
Number of animals	10	10	9	10	8
Necropsy body weight (g)	355 ± 19.1	357 ± 8.5 (+0.6%)	336 ± 23.1 (−5.4%)	342 ± 31.5 (−3.7%)	325 ± 26.8* (−8.5%)
Absolute liver weight (mg)	13,593 ± 2,121	15,661 ± 793 (+15%)	15,861 ± 1,712 (+17%)	18,742 ± 2,631** (+38%)	16,759 ± 2,660** (+23%)
Relative liver weight (mg/g)	38.4 ± 6.07	43.9 ± 1.87* (+14%)	47.2 ± 2.79** (+23%)	54.8 ± 5.08** (+43%)	51.3 ± 5.42** (+34%)
Female Rats					
Average daily dose, mg/kg-d	**0**	**134**	**268**	**536**	**1,071**
Number of animals	10	9	10	8	10
Necropsy body weight (g)	208 ± 15.9	200 ± 15.6 (−3.8%)	201 ± 11.3 (−3.4%)	191 ± 8.8* (−8.2%)	195 ± 12.1 (−6.3%)
Absolute liver weight (mg)	6,606 ± 795	7,818 ± 814** (+18%)	8,027 ± 688** (+22%)	7,988 ± 591** (+21%)	8,413 ± 652** (+27%)
Relative liver weight (mg/g)	31.8 ± 3.28	39.3 ± 3.53** (+24%)	39.9 ± 2.99** (+25%)	41.8 ± 2.81** (+31%)	43.2 ± 2.61** (+36%)

[a]NTP (1988).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
*Statistically significantly different from control at $p \leq 0.05$, as reported by the study authors (Dunnett's test).
**Statistically significantly different from control at $p \leq 0.01$, as reported by the study authors (Dunnett's test).

MBT = 2-mercaptobenzothiazole.

Table B-2. Mortality and Liver Weights in B6C3F$_1$ Mice Exposed by Gavage to MBT for 13 Weeks[a,b]

Male Rats						
Average daily dose, mg/kg-d	**0**	**67**	**134**	**268**	**536**	**1,071**
Mortality	0/10	0/10	0/10	0/10	0/10	5/10* (50%)
Necropsy body weight (g)	36.7 ± 2.8	37.0 ± 2.6 (+0.8%)	37.7 ± 3.1 (+2.7%)	35.1 ± 3.4 (−4.4%)	34.4 ± 2.0 (−6.3%)	35.2 ± 2.8 (−4.1%)
Absolute liver weight (mg)	1,821 ± 213	1,942 ± 208 (+7%)	2,034 ± 184 (+12%)	1,855 ± 231 (+2%)	1,809 ± 115 (−1%)	2,090 ± 184 (+15%)
Relative liver weight (mg/g)	49.6 ± 4.34	52.5 ± 4.02 (+5.8%)	54.0 ± 3.23* (+8.9%)	52.8 ± 3.51 (+6.5%)	52.6 ± 3.15 (+6.0%)	59.5 ± 3.94** (+20%)
Female Rats						
Average daily dose, mg/kg-d	**0**	**67**	**134**	**268**	**536**	**1,071**
Mortality	0/10	0/10	0/10	0/10	2/10 (20%)	7/10* (70%)
Necropsy body weight (g)	26.2 ± 1.3	25.5 ± 1.3 (−2.7%)	25.9 ± 1.7 (−1.1%)	25.8 ± 1.3 (−1.5%)	26.1 ± 1.3 (−0.4%)	25.3 ± 0.3 (−3.4%)
Absolute liver weight (mg)	1,129 ± 242	1,237 ± 123 (+9.6%)	1,238 ± 113 (+9.7%)	1,232 ± 124 (+9.1%)	1,281 ± 126 (+13%)	1,383 ± 96 (+22%)
Relative liver weight (mg/g)	42.9 ± 7.71	48.6 ± 5.03 (+13%)	47.9 ± 3.61 (+12%)	47.8 ± 3.74 (+11%)	49.2 ± 4.70 (+15%)	54.7 ± 3.45** (+28%)

[a]NTP (1988).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
*Statistically significantly different from control at $p \leq 0.05$, as reported by the study authors (Dunnett's test).
**Statistically significantly different from control at $p \leq 0.01$, as reported by the study authors (Dunnett's test).

MBT = 2-mercaptobenzothiazole.

Table B-3. Nonaccidental Deaths and Incidences of Selected Non-neoplastic Lesions in F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Male Rats				
Average daily dose, mg/kg-d	**0**	**134**	**268**	**536**
Nonaccidental deaths[b]	8/50 (16%)	NA	28/50** (56%)	29/49** (59%)
Forestomach				
Ulcer	0/50	NA	5/50* (10%)	5/49* (10%)
Inflammation	0/50	NA	11/50* (22%)	14/49* (29%)
Epithelial hyperplasia	1/50 (2%)	NA	12/50* (24%)	17/49* (35%)
Hyperkeratosis	0/50	NA	12/50* (24%)	17/49* (35%)
Kidney				
Nephropathy	50/50 (100%)	NA	50/50 (100%)	50/50 (100%)
Nephropathy severity (mean)	2.3	NA	3.4	3.4
Pelvis epithelial hyperplasia	0/50	NA	4/50 (8%)	1/49 (2%)
Tubule focal hyperplasia	0/50	NA	3/50 (6%)	3/49 (6%)
Female Rats				
Average daily dose, mg/kg-d	**0**	**134**	**268**	**536**
Nonaccidental deaths[b]	21/49 (43%)	18/49 (37%)	25/50 (50%)	NA
Forestomach				
Ulcer	0/49	3/50 (6%)	5/50* (10%)	NA
Inflammation	2/49 (4%)	4/50 (8%)	7/50 (14%)	NA
Epithelial hyperplasia	1/49 (2%)	4/50 (8%)	1/50 (2%)	NA
Hyperkeratosis	1/49 (2%)	4/50 (8%)	1/50 (2%)	NA
Kidney				
Nephropathy (severity not reported)	38/49 (76%)	42/50 (84%)	41/50 (82%)	NA

[a]NTP (1988).
[b]Excludes animals accidentally killed.
*Significantly different from control at $p < 0.05$ based on Fisher's exact test performed for this review.
**Significantly different from control at $p < 0.01$ based on life table test performed by the study authors.

MBT = 2-mercaptobenzothiazole; NA = not available.

Table B-4. Incidences of Selected Neoplastic Lesions in F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Male Rats				
HED, mg/kg-d	**0**	**64.3**	**129**	**Historical control incidence in NTP studies (corn oil gavage)**
Mononuclear cell leukemia	7/50 (14%)	16/50* (32%)	3/50 (6%)	202/1,450 (14%) Range: 1/50−14/50
Pituitary gland adenoma	14/50 (28%)	21/50* (42%)	12/48 (24%)	344/1,411 (8%) Range: 5/50−19/50
Pancreatic acinar cell adenoma	2/50[†] (4%)	13/50*[#] (26%)	6/49* (12%)	80/1,381 (8%) Range: 0/50−14/50
Mesothelioma	0/50[†]	2/50 (4%)	3/50 (6%)	55/1,450 (4%) Range: 0/50−6/50
Adrenal gland				
Pheochromocytoma	18/50[†] (36%)	25/50* (50%)	22/49* (45%)	338/1,442 (23%) Range: 2/50−20/49
Pheochromocytoma or malignant pheochromocytoma	18/50[†] (36%)	27/50*[#] (54%)	24/49*[#] (49%)	347/1,442 (24%) Range: 2/50−20/49
Preputial gland				
Adenoma	0/50[†]	4/50* (8%)	4/50* (8%)	30/1,450 (2%) Range: 0/50−7/50
Adenoma or carcinoma	1/50[†] (2%)	6/50* (12%)	5/50* (10%)	65/1,450 (4.5%) Range: 0/50−9/50
Subcutaneous tissue				
Fibroma	2/50[†] (4%)	3/50 (6%)	6/50* (12%)	93/1,450 (6.4%) Range: 0/50−6/50
Fibroma, neurofibroma, sarcoma, or fibrosarcoma	3/50[†] (6%)	6/50 (12%)	7/50* (14%)	126/1,450 (8.7%) Range: 1/50−8/50
Kidney				
Transitional cell papilloma	0/50	1/50 (2%)	1/49 (2%)	1/1,488 (0.07%) Range: NR
Transitional cell carcinoma	0/50	1/50 (2%)	0/49	NR
Tubular cell adenoma	0/50	1/50 (2%)	1/50 (2%)	3/1,488 (0.2%)

Table B-4. Incidences of Selected Neoplastic Lesions in F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Female Rats				
HED, mg/kg-d	**0**	**32.2**	**64.3**	**Historical control incidence in NTP studies (corn oil gavage)**
Pituitary gland				
Adenoma	15/49[†] (31%)	24/50 (48%)	25/50*[#] (50%)	520/1,407 (37%) Range: 9/50−27/49
Adenoma or adenocarcinoma	16/49[†] (33%)	24/50 (48%)	25/50*[#] (50%)	561/1,407 (39.9%) Range: 11/50−30/49
Adrenal gland, pheochromocytoma	1/50[†] (2%)	5/50 (10%)	6/50* (12%)	82/1,443 (5.7%) Range: 0/50−7/50

[a]NTP (1988).
*Significantly different from concurrent control at $p < 0.05$ based on life table test performed by the study authors.
[#]Significantly different from concurrent control at $p < 0.05$ based on incidental tumor test performed by the study authors.
[†]Significant ($p < 0.05$) dose-related trend by life table or incidental tumor analysis or both.

HED = human equivalent dose; MBT = 2-mercaptobenzothiazole; NR = not reported; NTP = National Toxicology Program.

Table B-5. Incidence of Nonaccidental Deaths Prior to Termination and Neoplastic Lesions in B6C3F$_1$ Mice Exposed by Gavage to MBT for 103 Weeks[a,b]

Male Mice			
Average daily dose, mg/kg-d (HED)	**0**	**268 (HED = 37.5)**	**536 (HED = 75.0)**
Mortality	11/50 (22%)	17/50 (34%)	14/44 (32%)
Hepatocellular adenoma or carcinoma (combined)	16/49 (33%)	21/50 (42%)	14/50 (28%)
Female Mice			
Average daily dose, mg/kg-d (HED)	**0**	**268 (HED = 37.5)**	**536 (HED = 75.0)**
Mortality	13/50[c] (26%)	10/49 (22%)	24/46** (52%)
Hepatocellular adenoma or carcinoma (combined)	4/50 (8%)	12/49* (24%)	4/50 (8%)

[a]NTP (1988).
[b]Excludes animals accidentally killed.
[c]Excludes two female vehicle control mice that died during termination period.
*Significantly different from control at $p < 0.05$ based on life table test performed by the study authors.
**Significantly different from control at $p < 0.01$ based on life table test performed by the study authors.

HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

Table B-6. Absolute and Relative Kidney and Liver Weights in F0 and F1 S-D Parents Exposed to MBT in the Diet[a]

Male Rats				
	Exposure group, mg/kg-d (*n* = 28)			
Parameter	**0**	**172.1**	**602.3**	**1,033**
F0 parents				
Terminal body weight (Wk 20) (g)	577 ± 52.1	561 ± 45.8 (−3%)	540 ± 48.4* (−6%)	530 ± 60.3* (−8%)
Absolute kidney weight (g)	4.28 ± 0.448[b]	4.39 ± 0.442 (+3%)	4.69 ± 0.470 (+10%)	4.70 ± 0.549 (+10%)
Relative kidney weight (g/100 g)	0.743 ± 0.0681	0.783 ± 0.0573 (+5%)	0.870 ± 0.0689* (+17%)	0.891 ± 0.0819* (+20%)
Absolute liver weight (g)	22.85 ± 2.914	22.22 ± 2.734 (−3%)	23.91 ± 2.609 (+5%)	24.56 ± 3.249 (+7%)
Relative liver weight (g/100 g)	3.956 ± 0.3412	3.955 ± 0.3288 (0%)	4.429 ± 0.3002* (+12%)	4.636 ± 0.3355* (+17%)
F1 parents				
Terminal body weight (Wk 38) (g)	627 ± 59.7	609 ± 52.5 (−3%)	584 ± 54.9* (−7%)	572 ± 70.0* (−9%)
Absolute kidney weight (g)	4.55 ± 0.511	4.67 ± 0.483 (+3%)	4.92 ± 0.667 (+8%)	4.98 ± 0.594* (+9%)
Relative kidney weight (g/100 g)	0.720 ± 0.0707	0.761 ± 0.0782 (+6%)	0.831 ± 0.0682* (+15%)	0.862 ± 0.0566* (+20%)
Absolute liver weight (g)	22.77 ± 3.027	24.77 ± 3.394 (+9%)	26.33 ± 3.365* (+16%)	27.74 ± 4.391* (+22%)
Relative liver weight (g/100 g)	3.591 ± 0.3104	4.018 ± 0.4010* (+12%)	4.451 ± 0.3339* (+24%)	4.783 ± 0.3447* (+33%)
Female Rats				
	Exposure group, mg/kg-d (*n* = 28)			
Parameter	**0**	**199.7**	**699.0**	**1,198**
F0 parents				
Terminal body weight (Wk 20) (g)	354 ± 32.3	347 ± 32.4 (−2%)	336 ± 21.8* (−5%)	329 ± 22.6* (−7%)
Absolute kidney weight (g)	2.87 ± 0.301	2.97 ± 0.296 (+3%)	3.00 ± 0.319 (+5%)	3.07 ± 0.299 (+7%)
Relative kidney weight (g/100 g)	0.812 ± 0.0559	0.860 ± 0.0753 (+6%)	0.892 ± 0.0735* (+10%)	0.935 ± 0.0862* (+15%)
Absolute liver weight (g)	15.84 ± 1.989	15.77 ± 1.834 (0%)	16.92 ± 1.633 (+7%)	16.96 ± 2.144 (+7%)
Relative liver weight (g/100 g)	4.471 ± 0.399	4.549 ± 0.4051 (+2%)	5.033 ± 0.3028* (+13%)	5.149 ± 0.4241* (+15%)

Table B-6. Absolute and Relative Kidney and Liver Weights in F0 and F1 S-D Parents Exposed to MBT in the Diet[a]

F1 parents				
Terminal body weight (Wk 38) (g)	324 ± 28.3	312 ± 20.3 (−4%)	304 ± 28.3* (−6%)	302 ± 21.3* (−7%)
Absolute kidney weight (g)	2.74 ± 0.311	2.82 ± 0.178 (+3%)	2.87 ± 0.310 (+5%)	2.82 ± 0.251 (+3%)
Relative kidney weight (g/100 g)	0.837 ± 0.0739	0.894 ± 0.0666* (+7%)	0.936 ± 0.0649* (+12%)	0.942 ± 0.0659* (+13%)
Absolute liver weight (g)	13.31 ± 1.912	13.73 ± 1.953 (+3%)	14.35 ± 1.694 (+8%)	14.80 ± 1.648 (+11%)
Relative liver weight (g/100 g)	4.071 ± 0.5640	4.342 ± 0.49 (+7%)	4.678 ± 0.4617* (+15%)	4.932 ± 0.4149* (+21%)

[a]Springborn Laboratories (1990b).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
*Statistically significantly different from control at $p \leq 0.05$, as reported by the study authors (Dunnett's test).

MBT = 2-mercaptobenzothiazole; S-D = Sprague-Dawley.

Table B-7. Incidence of Histopathological Findings in the Kidneys and Liver of F0 and F1 S-D Parents Exposed to MBT in the Diet[a]

Male Rats				
	Exposure Group, mg/kg-d			
Parameter	**0**	**172.1**	**602.3**	**1,033**
F0 parents				
α2u-g inclusions, tubules	4/28 (14%)	10/28* (36%)	8/28 (29%)	13/28* (46%)
Kidney, basophilic tubules, cortex	2/28 (7%)	9/28* (32%)	10/28* (36%)	13/28* (46%)
Kidney, pigment, brown	0/28	0/28	12/28* (43%)	17/28* (61%)
F1 parents				
α2u-g inclusions, tubules	4/28 (14%)	13/28* (46%)	10/28* (36%)	14/28* (50%)
Kidney, basophilic tubules, cortex	0/28	3/28 (11%)	4/28 (14%)	10/28* (36%)
Kidney, pigment, brown	0/28	0/28	13/28* (46%)	20/28* (71%)
Liver, hepatocyte hypertrophy	0/28	1/28 (4%)	22/28* (79%)	23/28* (82%)
Female Rats				
	Exposure Group, mg/kg-d			
Parameter	**0**	**199.7**	**699.0**	**1,198**
F0 parents				
Kidney, basophilic tubules, cortex	0/28	0/28	0/28	0/28
Kidney, pigment, brown	0/28	0/28	1/28 (4%)	4/28 (14%)
F1 parents				
Kidney, basophilic tubules, cortex	1/28 (4%)	0/28	1/28 (4%)	2/28 (7%)
Kidney, pigment, brown	0/28	0/28	0/28	6/28* (21%)
Liver, hepatocyte hypertrophy	0/28	0/28	5/28* (18%)	10/28* (36%)

[a]Springborn Laboratories (1990b).
*Statistically significantly different from control at $p \leq 0.05$, as reported by the study authors (Dunnett's test).

α2u-g = alpha 2u-globulin; MBT = 2-mercaptobenzothiazole; S-D = Sprague-Dawley.

Table B-8. Body Weights in F1 and F2 S-D Pups Exposed to MBT via Lactation (PNDs 1−21) and in the Diet (PNDs 15−21)[a]

Parameter	Maternal Dose, mg/kg-d (*n* = 23 or 27)			
	0	199.7	699.0	1,198
F1 pups				
LD 7	16.8 ± 1.93	16.2 ± 2.17 (−4%)	15.7 ± 2.50 (−7%)	15.1 ± 1.74 (−10%)
LD 14	34.7 ± 2.45	33.4 ± 3.06 (−4%)	31.9 ± 3.30* (−8%)	29.8 ± 3.07* (−14%)
LD 21	56.1 ± 3.89	53.0 ± 4.87 (−6%)	49.1 ± 4.70* (−12%)	44.4 ± 4.52* (−21%)
F2 pups				
LD 7	16.0 ± 1.75	14.9 ± 1.65 (−7%)	15.3 ± 1.78 (−4%)	14.8 ± 1.55 (−10%)
LD 14	34.4 ± 2.59	31.4 ± 2.62* (−9%)	31.2 ± 2.33* (−9%)	29.3 ± 2.12* (−14%)
LD 21	55.6 ± 4.18	50.6 ± 3.63* (−9%)	49.0 ± 4.11* (−12%)	44.2 ± 3.01* (−21%)

[a]Springborn Laboratories (1990b).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
*Statistically significantly different from control at $p \leq 0.01$, as reported by the study authors (Dunnett's test).

LD = lactation day; MBT = 2-mercaptobenzothiazole; PND = postnatal day; S-D = Sprague-Dawley.

Table B-9. Occurrence of Selected Clinical Signs in Pregnant Rats Exposed by Gavage to MBT on GDs 6−15[a]

Parameter	Exposure Group, mg/kg-d			
	0	300	1,200	1,800
Daily observations[b]				
Urine stain	10 (8)	0 (0)	6 (3)	35 (11)
Dark material around nose	2 (2)	3 (3)	1 (1)	5 (4)
Dark material around mouth	0	0	0	8 (5)
Postdose observations[b]				
Salivation	0	0	38 (17)	35 (15)
Urine stain	0	0	7 (5)	4 (4)
Dark material around mouth	0	1 (1)	15 (12)	10 (9)
Activity decreased	0	0	0	7 (5)

[a]Springborn Laboratories (1989e).
[b]Data reported as total number of occurrences (number of animals with at least one occurrence).

GD = gestation day; MBT = 2-mercaptobenzothiazole.

Table B-10. Summary of Weight and Food Consumption Data for Pregnant Rats Exposed by Gavage to MBT on GDs 6−15[a]

Parameter		Exposure Group, mg/kg-d			
		0	**300**	**1,200**	**1,800**
Mean body weight (g)[b]	D 0	281 ± 19.3	279 ± 15.9 (−0.7%)	277 ± 14.9 (−1.4%)	277 ± 13.2 (−1.4%)
	D 6	318 ± 21.9	314 ± 18.7 (−1.3%)	315 ± 16.0 (−0.9%)	317 ± 18.0 (−0.3%)
	D 9	322 ± 21.4	321 ± 16.5 (−0.3%)	322 ± 18.2 (0)	308 ± 18.2* (−4.3%)
	D 12	340 ± 23.7	342 ± 16.5 (+0.6%)	341 ± 19.7 (+0.3%)	333 ± 28.0 (−2.1%)
	D 16	367 ± 29.4	372 ± 18.7 (+1.4%)	366 ± 22.8 (−0.3)	364 ± 24.7 (−0.8%)
	D 20	435 ± 40.9	445 ± 22.6 (+2.3%)	433 ± 28.3 (−0.5)	429 ± 32.7 (−1.4%)
Mean food intake (g/kg-d)[b]	D 0−6	83 ± 6.5	84 ± 5.9 (+1.2%)	85 ± 5.2 (+2.4%)	87 ± 5.6 (+4.8%)
	D 6−9	52 ± 5.1	54 ± 5.3 (+3.8%)	52 ± 8.4 (0)	38 ± 10.9** (−27%)
	D 9−12	57 ± 4.8	61 ± 4.6 (+7.0%)	63 ± 5.6* (+11%)	65 ± 15.5** (+14%)
	D 12−16	57 ± 6.6	59 ± 3.7 (+3.5%)	59 ± 5.2 (+3.5%)	61 ± 9.6 (+7.0%)
	D 16−20	74 ± 6.1	72 ± 3.1 (−2.7%)	72 ± 3.9 (−2.7%)	74 ± 5.3 (0)

[a]Springborn Laboratories (1989e).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean)/control mean] × 100.
*Significantly different from control at $p < 0.05$, as reported by the study authors (Dunnett's test).
**Significantly different from control at $p < 0.01$, as reported by the study authors (Dunnett's test).

GD = gestation day; MBT = 2-mercaptobenzothiazole.

Table B-11. Selected Developmental Effects in Rats Exposed by Gavage to MBT on GDs 6−15[a]

Parameter		Exposure Group, mg/kg-d			
		0	**300**	**1,200**	**1,800**
Number of females gravid		24 (92.3%)	23 (88.5%)	25 (96.2%)	22 (84.6%)
Corpora lutea[b]		18.0 ± 3.4	17.8 ± 2.2 (−1%)	17.6 ± 2.6 (−2%)	18.8 ± 2.1 (+4%)
Implantation sites[b]		14.8 ± 4.0	16.6 ± 1.4 (+12%)	15.8 ± 3.2 (+7%)	16.6 ± 3.2 (+12%)
Preimplantation loss[b]		3.2 ± 5.4	1.2 ± 1.7 (−63%)	1.8 ± 3.2 (−44%)	2.2 ± 3.8 (−31%)
Postimplantation loss[b,c]		0.8 ± 0.9	1.3 ± 0.9* (+63%)	1.3 ± 1.2 (+63%)	1.7 ± 1.7* (+113%)
Early resorptions[b]		0.8 ± 0.9	1.3 ± 0.9* (+63%)	1.3 ± 1.2 (+63%)	1.6 ± 1.7 (+100%)
Late resorptions[b]		0	0	0	0 ± 0.2 (n = 1)
Dead fetuses		0	0	0	0
Viable fetuses[b]		14.0 ± 3.8	15.3 ± 2.0 (+9%)	14.5 ± 3.2 (+4%)	14.9 ± 3.1 (+6%)
Sex[b]	Male	7.7 ± 2.8	7.9 ± 2.3 (+3%)	7.6 ± 2.5 (−1%)	7.1 ± 2.7 (−8%)
	Female	6.3 ± 2.5	7.3 ± 2.4 (+16%)	6.9 ± 2.7 (+10%)	7.8 ± 3.0 (+24%)
Fetal weight (g)[b]		3.7 ± 0.5	3.6 ± 0.2 (−3%)	3.7 ± 0.2 (0)	3.5 ± 0.3 (−5%)

[a]Springborn Laboratories (1989e).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Historical control range in the laboratory: 0.6−1.4 per litter; mean: 0.9 per litter.
*Significantly different from control at $p < 0.05$, as reported by the study authors (Dunnett's test).

GD = gestation day; MBT = 2-mercaptobenzothiazole.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

MODELING OF NONCANCER ENDPOINTS

As discussed in the body of the report in the "Derivation of Oral Reference Doses" section, the endpoints selected for benchmark dose (BMD) modeling were increased relative and absolute liver weight in male and female rats and female mice exposed to 2-mercaptobenzothiazole (MBT) for 13 weeks by gavage (NTP, 1988). The incidence data used in the modeling are shown in Tables 8 and C-1.

Table C-1. Selected Non-neoplastic Endpoints in F344 Rats and B6C3F$_1$ Mice Exposed by Gavage to MBT for 13 Weeks[a,b]

Male Rats					
Average daily dose, mg/kg-d	**0**	**134**	**268**	**536**	**1,071**
Number of animals	10	10	9	10	8
Absolute liver weight (mg)	13,593 ± 2,121	15,661 ± 793 (+15%)	15,861 ± 1,712 (+17%)	18,742 ± 2,631** (+38%)	16,759 ± 2,660** (+23%)
Relative liver weight (mg/g)	38.4 ± 6.07	43.9 ± 1.87* (+14%)	47.2 ± 2.79** (+23%)	54.8 ± 5.08** (+43%)	51.3 ± 5.42** (+34%)
Female Rats					
Average daily dose, mg/kg-d	**0**	**134**	**268**	**536**	**1,071**
Number of animals	10	9	10	8	10
Absolute liver weight (mg)	6,606 ± 795	7,818 ± 814** (+18%)	8,027 ± 688** (+22%)	7,988 ± 591** (+21%)	8,413 ± 652** (+27%)
Relative liver weight (mg/g)	31.8 ± 3.28	39.3 ± 3.53** (+24%)	39.9 ± 2.99** (+25%)	41.8 ± 2.81** (+31%)	43.2 ± 2.61** (+36%)

Female Mice						
Average daily dose, mg/kg-d	**0**	**67**	**134**	**268**	**536**	**1,071**
Number of animals	10	10	10	10	8	3[c]
Absolute liver weight (mg)	1,129 ± 242	1,237 ± 123 (+9.6%)	1,238 ± 113 (+9.7%)	1,232 ± 124 (+9.1%)	1,281 ± 126 (+13%)	1,383 ± 96 (+22%)
Relative liver weight (mg/g)	42.9 ± 7.71	48.6 ± 5.03 (+13%)	47.9 ± 3.61 (+12%)	47.8 ± 3.74 (+11%)	49.2 ± 4.70 (+15%)	54.7 ± 3.45** (+28%)

[a]NTP (1988).
[b]Values expressed as mean ± standard deviation (% change compared with control); % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Statistically significant mortality (7/10).
*Statistically significantly different from control at $p \leq 0.05$, as reported by the study authors (Dunnett's test).
**Statistically significantly different from control at $p \leq 0.01$, as reported by the study authors (Dunnett's test).

MBT = 2-mercaptobenzothiazole.

MODELING PROCEDURE FOR CONTINUOUS DATA

BMD modeling of continuous noncancer data was conducted with the EPA's Benchmark Dose Software (BMDS, Version 2.5). For these data, all continuous models available within the software were fit using a benchmark response (BMR) of 10% extra risk or 1 standard deviation (SD). Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals at the data point (except the control) closest to the predefined BMR (absolute value <2.0), and visual inspection of the model fit. In addition to these three criteria for judging the adequacy of model fit, a determination was made as to whether the variance across dose groups was homogeneous. If a homogeneous variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from the homogeneous variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model did not adequately fit the data (i.e., Test 3; $p < 0.1$), the data set was considered unsuitable for BMD modeling. In the cases where no best model was found to fit to the data, a reduced data set without the high-dose group was further attempted for modeling and the result was present along with that of the full data set. Among all of the models providing adequate fit, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential point of departure (POD) when BMDL values were sufficiently close. Otherwise, the lowest BMDL was selected as a potential POD.

Model Predictions for Increased Absolute Liver Weight in Male Rats

The procedure outlined above was applied to the data on increased absolute liver weight in male rats (see Table C-1) (NTP, 1988). Neither the constant nor the nonconstant variance models in the BMDS provided adequate fit to the variance data; thus, these data were not suitable for BMD modeling (see Table C-2).

Table C-2. Modeling Results for Increased Absolute Liver Weights in Male F344 Rats Administered MBT via Gavage for 13 Weeks[a,g]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals for Dose Group[d]	AIC
Constant variance, all doses					
Exponential (Model 2)[e]	<0.0001	0.008339	0.0003809	2.952	784.1144
Exponential (Model 3)[e]	<0.0001	0.008339	0.0003809	2.952	784.1144
Exponential (Model 4)[e]	<0.0001	0.008339	0.02882	0.1036	774.9049
Exponential (Model 5)[e]	<0.0001	0.008339	0.009944	0.6077	776.4563
Hill[e]	<0.0001	0.008339	0.006453	0.399	777.230503
Linear[f]	<0.0001	0.008339	0.000508	2.88	783.507998
Polynomial (2-degree)[f]	<0.0001	0.008339	0.000508	2.88	783.507994
Polynomial (3-degree)[f]	<0.0001	0.008339	0.0005077	2.88	783.509001
Power[e]	<0.0001	0.008339	0.000508	2.88	783.507994
Nonconstant variance, all doses					
Linear[f]	<0.0001	0.006591	0.002715	0.212	780.506214
Constant variance, highest dose dropped					
Linear[f]	<0.0001	0.006376	0.4309	0.986	633.007758
Nonconstant variance, highest dose dropped					
Linear[f]	<0.0001	0.003587	0.5179	1.08	633.585511

[a]NTP (1988).
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals at doses closest to BMD and the largest residual at any dose.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.
[g]No model was selected. Neither the constant nor nonconstant variance models provide adequate fit to the variance data using either the full or reduced dataset.

AIC = Akaike's information criterion; BMD = benchmark dose; MBT = 2-mercaptobenzothiazole.

Model Predictions for Increased Relative Liver Weight in Male Rats

The procedure outlined above was applied to the data on increased relative liver weight in male rats (see Table C-1) (NTP, 1988). Neither the constant nor the nonconstant variance models in the BMDS provided adequate fit to the variance data; thus, these data were not suitable for BMD modeling (see Table C-3).

Table C-3. Modeling Results for Increased Relative Liver Weights in Male F344 Rats Administered MBT via Gavage for 13 Weeks[a,g]

Model	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	Scaled Residuals for Dose Group[d]	AIC
Constant variance, all doses					
Exponential (Model 2)[e]	<0.0001	0.003799	<0.0001	3.396	218.362
Exponential (Model 3)[e]	<0.0001	0.003799	<0.0001	3.396	218.362
Exponential (Model 4)[e]	<0.0001	0.003799	0.02857	0.3174	198.9066
Exponential (Model 5)[e]	<0.0001	0.003799	0.0288	0.5853	198.5753
Hill[e]	<0.0001	0.003799	0.01187	0.463	200.126363
Linear[f]	<0.0001	0.003799	<0.0001	0.833	216.60979
Polynomial (2-degree)[f]	<0.0001	0.003799	<0.0001	0.833	216.60979
Polynomial (3-degree)[f]	<0.0001	0.003799	<0.0001	0.833	216.60979
Power[e]	<0.0001	0.003799	<0.0001	0.833	216.60979
Nonconstant variance, all doses					
Linear[f]	<0.0001	0.001502	<0.0001	1.02	216.517628
Constant variance, highest dose dropped					
Linear[f]	<0.0001	0.001962	0.7012	0.625	155.886575
Nonconstant variance, highest dose dropped					
Linear[f]	<0.0001	0.0007209	0.629	0.607	157.73721

[a]NTP (1988).
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals at doses closest to BMD and the largest residual at any dose.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.
[g]No model was selected. Neither the constant nor nonconstant variance models provide adequate fit to the variance data using either the full or reduced dataset.

AIC = Akaike's information criterion; BMD = benchmark dose; MBT = 2-mercaptobenzothiazole.

Model Predictions for Increased Absolute Liver Weight in Female Rats

The procedure outlined above was applied to the data on increased absolute liver weight in female rats (NTP, 1988) (see Table C-1). The constant variance model provided adequate fit to the variance data ($p > 0.1$). With the constant variance model applied, the Exponential (Models 4 and 5) and Hill models provided adequate fit to the means data. BMDL values for models providing adequate fit differed by four-fold. However, the ratio of BMD to BMDL values is very large (>100-fold) for all models that provide an adequate fit. For this reason, the data are not suitable for BMD modeling (see Table C-4).

Table C-4. Modeling Results for Increased Absolute Liver Weights in Female F344 Rats Administered MBT via Gavage for 13 Weeks[a]

Model	BMD	$BMDL_{10}$	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	AIC
Constant variance, all doses						
Exponential (Model 2)[e]	626.618	448.444	0.0001553	0.8587	0.00171	680.7429
Exponential (Model 3)[e]	626.618	448.444	0.0001553	0.8587	0.00171	680.7429
Exponential (Model 4)[e]	56.8777	0.228986	0.0001553	0.8587	0.4105	669.3949
Exponential (Model 5)[e]	56.8776	0.23809	0.0001553	0.8587	0.4105	669.3949
Hill[e]	42.4377	3.06×10^{-5}	0.0001553	0.8587	0.5348	668.865905
Linear[f]	585.717	406.056	0.0001553	0.8587	0.002113	680.292823
Polynomial (2-degree)[f]	585.662	406.158	0.0001553	0.8587	0.002117	680.288636
Polynomial (3-degree)[f]	585.671	405.964	0.0001553	0.8587	0.002109	680.296611
Power[e]	585.661	406.158	0.0001553	0.8587	0.002117	680.288635

[a]NTP (1988).
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals at doses closest to BMD and the largest residual at any dose.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); MBT = 2-mercaptobenzothiazole.

Model Predictions for Increased Relative Liver Weight in Female Rats

The procedure outlined above was applied to the data on increased relative liver weight in female rats (NTP, 1988) (see Table C-1). Table C-5 summarizes the BMD modeling results. The constant variance model provided adequate fit to the variance data. With the constant variance model applied, the Exponential (Models 4 and 5) and Hill models provided adequate fit to the means data ($p > 0.1$). BMDLs for models providing adequate fit were sufficiently close (differed by less than two to threefold), so the model with the lowest AIC was selected (Hill). Visual inspection of the shape of the Hill dose-response curve in the low-dose region appears to be reasonable. Thus, the $BMDL_{10}$ of 14.8 mg/kg-day from this model is selected for this endpoint (see Figure C-1 and the BMD text output for details).

Table C-5. Modeling Results for Increased Relative Liver Weights in Female F344 Rats Administered MBT via Gavage for 13 Weeks[a]

Model	BMD	$BMDL_{10}$	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	AIC
Constant variance, all doses						
Exponential (Model 2)[e]	483.317	374.461	<0.0001	0.8968	<0.0001	179.8442
Exponential (Model 3)[e]	483.317	374.461	<0.0001	0.8968	<0.0001	179.8442
Exponential (Model 4)[e]	52.3996	27.9336	<0.0001	0.8968	0.2475	157.6713
Exponential (Model 5)[e]	52.3996	27.9336	<0.0001	0.8968	0.2475	157.6713
Hill[e]	**36.2984**	**14.8244**	**<0.0001**	**0.8968**	**0.5774**	**155.97703**
Linear[f]	434.701	327.334	<0.0001	0.8968	<0.0001	178.674032
Polynomial (2-degree)[f]	434.7	327.334	<0.0001	0.8968	<0.0001	178.674032
Polynomial (3-degree)[f]	434.701	327.334	<0.0001	0.8968	<0.0001	178.674032
Power[e]	434.701	327.334	<0.0001	0.8968	<0.0001	178.674032

[a]NTP (1988).
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Scaled residuals at doses closest to BMD and the largest residual at any dose.
[e]Power restricted to ≥1.
[f]Coefficients restricted to be negative.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); MBT = 2-mercaptobenzothiazole.

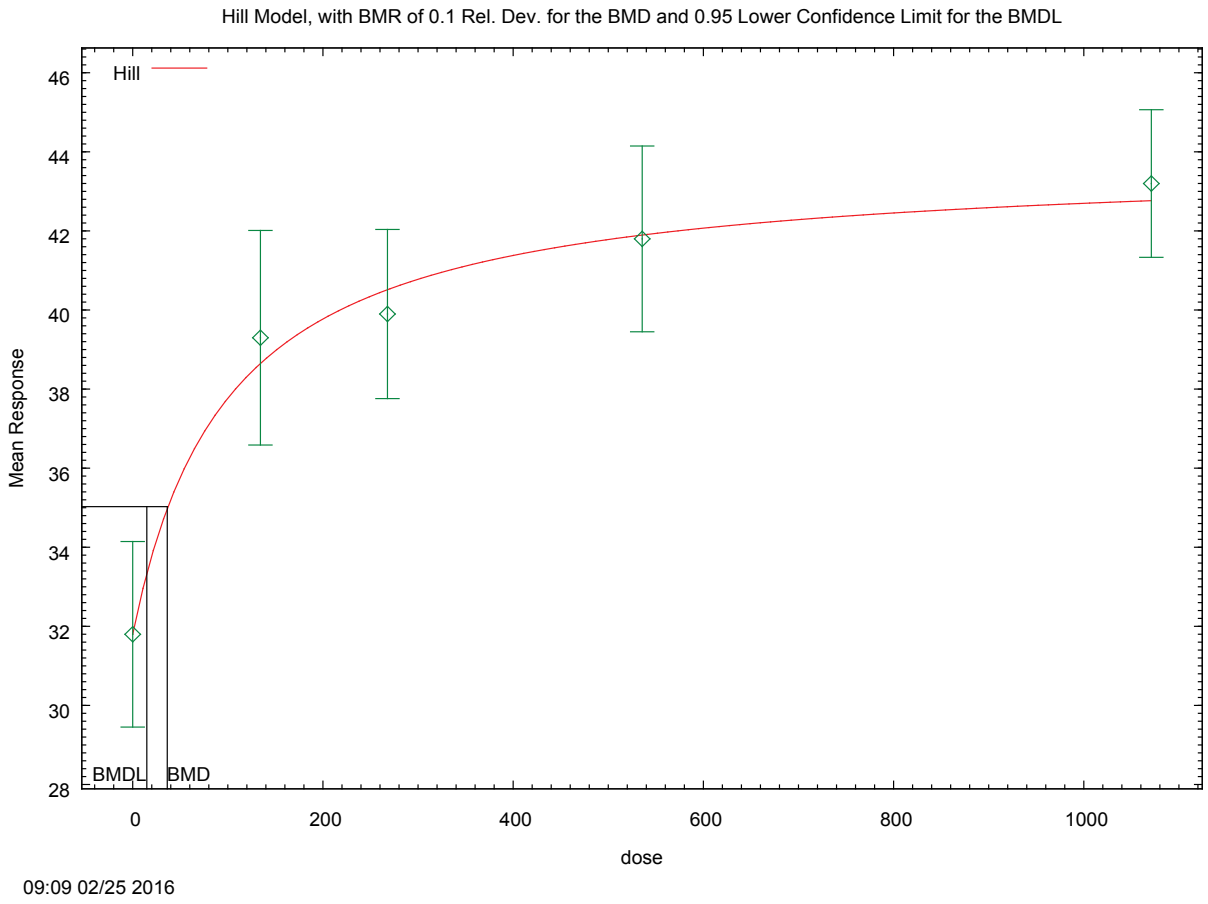

Figure C-1. Hill Model for Increased Relative Liver Weight in Female Rats Administered MBT via Gavage for 13 Weeks (NTP, 1988)

Text Output for Hill Model for Increased Relative Liver Weight in Female Rats Administered MBT via Gavage for 13 Weeks (NTP, 1988)

```
 ================================================================
        Hill Model. (Version: 2.17;  Date: 01/28/2013)
        Input Data File: C:/Users/bowens/BMDS2601/Data/hil_Continuous1_Opt.(d)
        Gnuplot Plotting File:  C:/Users/bowens/BMDS2601/Data/hil_Continuous1_Opt.plt
                                          Thu Feb 25 09:09:01 2016
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
```

Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	9.37036	
rho =	0	Specified
intercept =	31.8	
v =	11.4	
n =	1.19055	
k =	166.52	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	k
alpha	1	-5e-009	1.4e-007	9.7e-008
intercept	-5e-009	1	-0.54	0.32
v	1.4e-007	-0.54	1	0.52
k	9.7e-008	0.32	0.52	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	8.57154	1.76817	5.10598	12.0371
intercept	31.8439	0.929732	30.0216	33.6661
v	12.0427	1.49767	9.10735	14.9781
n	1	NA		
k	100.975	49.6702	3.62317	198.327

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.

0	10	31.8	31.8	3.28	2.93	-0.0474
134.3	9	39.3	38.7	3.53	2.93	0.596
267.9	10	39.9	40.6	2.99	2.93	-0.745
535.7	8	41.8	42	2.81	2.93	-0.171
1071	10	43.2	42.8	2.61	2.93	0.379

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-73.439239	6	158.878478
A2	-72.897270	10	165.794540
A3	-73.439239	6	158.878478
fitted	-73.988515	4	155.977030
R	-98.834106	2	201.668212

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	51.8737	8	<.0001
Test 2	1.08394	4	0.8968
Test 3	1.08394	4	0.8968
Test 4	1.09855	2	0.5774

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

```
The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =     Relative deviation

Confidence level =           0.95

             BMD =        36.2984

            BMDL =       14.8244
```

Model Predictions for Increased Absolute Liver Weight in Female Mice

The procedure outlined above was applied to the data on increased absolute liver weight in female mice (NTP, 1988) (see Table C-1). The constant variance model did not provide adequate fit to the variance data ($p < 0.1$). The nonconstant variance model did provide an adequate fit to the variance data ($p > 0.1$). The Exponential (2- and 3-degree), Hill, Linear, Polynomial (2- and 3-degree), and Power models provided adequate fit to the means data. However, visual inspection of the plots showed the Exponential (2- and 3-degree), Linear, Polynomial, and Power models did not provide a good fit to the data in the low dose range. In addition, the ratio of BMD to BMDL values provided by the Hill model is very large (>100-fold). Removing the high-dose group did not improve the model fit. For these reasons, the data were determined to be not suitable for BMD modeling (see Table C-6).

Table C-6. Modeling Results for Increased Absolute Liver Weights in Female Mice Administered MBT via Gavage for 13 Weeks[a]

Model	BMD	$BMDL_{10}$	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	AIC
Constant variance, all doses						
Exponential (Model 2)[e]	636.589	391.993	0.03112	0.04899	0.6167	566.0242
Exponential (Model 3)[e]	636.589	391.993	0.03112	0.04899	0.6167	566.0242
Exponential (Model 4)[e]	458.073	2.43592	0.03112	0.04899	0.4967	567.7505
Exponential (Model 5)[e]	458.072	1.92029	0.03112	0.04899	0.4967	567.7505
Hill[e]	166.825	0.00093185	0.03112	0.04899	0.5739	567.359494
Linear[f]	615.628	361.926	0.03112	0.04899	0.6261	565.97082
Polynomial (2-degree)[f]	615.634	361.926	0.03112	0.04899	0.6261	565.97082
Polynomial (3-degree)[f]	615.642	361.926	0.03112	0.04899	0.6261	565.97082
Power[e]	615.64	361.926	0.03112	0.04899	0.6261	565.97082
Nonconstant variance, all doses						
Exponential (Model 2)[d]	672.57	464.652	0.03112	0.9393	0.1024	562.7552
Exponential (Model 3)[d]	672.571	464.652	0.03112	0.9393	0.1024	562.7552
Exponential (Model 4)[d]	426.345	1.5383	0.03112	0.9393	0.07711	563.8785
Exponential (Model 5)[d]	426.345	1.60125	0.03112	0.9393	0.07711	563.8785
Hill[e]	55.6028	1.07×10^{-12}	0.03112	0.9393	0.232	561.324452
Linear[f]	646.418	430.352	0.03112	0.9393	0.109	562.599204
Polynomial (2-degree)[e]	646.418	430.352	0.03112	0.9393	0.109	562.599204
Polynomial (3-degree)[e]	646.418	430.352	0.03112	0.9393	0.109	562.599204
Power[e]	646.418	430.352	0.03112	0.9393	0.109	562.599204

Table C-6. Modeling Results for Increased Absolute Liver Weights in Female Mice Administered MBT via Gavage for 13 Weeks[a]

Model	BMD	$BMDL_{10}$	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	AIC
Nonconstant variance, high dose dropped						
Exponential (Model 2)[d]	654.21	340.709	0.06175	0.889	0.05397	533.3498
Exponential (Model 3)[d]	654.21	340.709	0.06175	0.889	0.05397	533.3498
Exponential (Model 4)[d]	68.5973	0.114258	0.06175	0.889	0.8062	528.1367
Exponential (Model 5)[d]	34.0961	0.112673	0.06175	0.889	0.491	530.1801
Hill[e]	63.5382	3.10×10^{-5}	0.06175	0.889	0.8195	528.103897
Linear[f]	645.758	325.109	0.06175	0.889	0.05658	533.244022
Polynomial (2-degree)[e]	645.755	325.109	0.06175	0.889	0.05658	533.244022
Polynomial (3-degree)[e]	645.755	325.109	0.06175	0.889	0.05658	533.244022
Power[e]	645.756	325.109	0.06175	0.889	0.05658	533.244022

[a]NTP (1988).
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Power restricted to ≥1.
[e]Coefficients restricted to be negative.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); MBT = 2-mercaptobenzothiazole.

Model Predictions for Increased Relative Liver Weight in Female Mice

The procedure outlined above was applied to the data on increased relative liver weight in female mice (NTP, 1988) (see Table C-1). The constant variance model did not provide an adequate fit to the variance data ($p < 0.1$). The nonconstant variance model did provide an adequate fit to the variance data ($p > 0.1$), but not the means data ($p < 0.1$). After dropping the high-dose group and applying the nonconstant variance model, the Exponential (Models 4 and 5) and Hill models provided adequate fit to the means data. BMDLs for models providing adequate fit differed by more than 100-fold. In addition, the ratio of BMD to BMDL values is very large (>100-fold) for all models that provide an adequate fit. For these reasons, the data were not suitable for BMD modeling (see Table C-7).

Table C-7. Modeling Results for Increased Relative Liver Weights in Female Mice Administered MBT via Gavage for 13 Weeks[a]

Model	BMD	$BMDL_{10}$	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	AIC
Constant variance, all doses						
Exponential (Model 2)[d]	580.301	384.207	0.006477	0.09094	0.1836	223.6164
Exponential (Model 3)[d]	580.301	384.207	0.006477	0.09094	0.1836	223.6164
Exponential (Model 4)[d]	459.826	2.99338	0.006477	0.09094	0.1088	225.4588
Exponential (Model 5)[d]	459.826	2.5459	0.006477	0.09094	0.1088	225.4588
Hill[e]	55.7046	0.000271543	0.006477	0.09094	0.2188	223.827617
Linear[f]	558.964	354.726	0.006477	0.09094	0.1869	223.568261
Polynomial (2-degree)[e]	558.964	354.726	0.006477	0.09094	0.1869	223.568261
Polynomial (3-degree)[e]	558.964	354.726	0.006477	0.09094	0.1869	223.568261
Power[e]	558.964	354.726	0.006477	0.09094	0.1869	223.568261
Nonconstant variance, all doses						
Exponential (Model 2)[d]	587.092	432.887	0.006477	0.701	0.06265	221.0346
Exponential (Model 3)[d]	587.092	432.887	0.006477	0.701	0.06265	221.0346
Exponential (Model 4)[d]	494.184	1.81331	0.006477	0.701	0.03298	222.8339
Exponential (Model 5)[d]	494.183	2.1962	0.006477	0.701	0.03298	222.8339
Hill[e]	41.3349	1.07×10^{-12}	0.006477	0.701	0.09193	220.53908
Linear[f]	561.733	399.693	0.006477	0.701	0.065	220.944482
Polynomial (2-degree)[e]	561.733	399.693	0.006477	0.701	0.065	220.944482
Polynomial (3-degree)[e]	564.197	399.698	0.006477	0.701	0.03138	222.944257
Power[e]	561.733	399.693	0.006477	0.701	0.065	220.944482

Table C-7. Modeling Results for Increased Relative Liver Weights in Female Mice Administered MBT via Gavage for 13 Weeks[a]

Model	BMD	$BMDL_{10}$	Test for Significant Difference *p*-Value[b]	Variance *p*-Value[c]	Means *p*-Value[c]	AIC
Nonconstant variance, high dose dropped						
Exponential (Model 2)[d]	710.433	369.19	0.02619	0.5628	0.03248	211.5112
Exponential (Model 3)[d]	710.433	369.19	0.02619	0.5628	0.03248	211.5112
Exponential (Model 4)[d]	41.711	0.102377	0.02619	0.5628	0.9504	204.841
Exponential (Model 5)[d]	41.7109	0.159579	0.02619	0.5628	0.9504	204.841
Hill[e]	25.1861	5.36×10^{-13}	0.02619	0.5628	0.9601	204.820687
Linear[f]	701.798	354.147	0.02619	0.5628	0.03428	211.39192
Polynomial (2-degree)[e]	701.798	354.147	0.02619	0.5628	0.03428	211.39192
Polynomial (3-degree)[e]	701.797	354.147	0.02619	0.5628	0.03428	211.39192
Power[e]	701.798	354.147	0.02619	0.5628	0.03428	211.39192

[a]NTP (1988).
[b]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[d]Power restricted to ≥1.
[e]Coefficients restricted to be negative.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); MBT = 2-mercaptobenzothiazole.

MODELING OF CANCER ENDPOINTS

As discussed in the body of the report in the "Derivation of a Provisional Oral Slope Factor" section, the tumor types selected for BMD modeling were mesothelioma, adrenal gland pheochromocytoma or malignant pheochromocytoma, preputial gland adenoma or carcinoma, and subcutaneous tissue fibroma, neurofibroma, sarcoma, or fibrosarcoma in male rats (NTP, 1988), and adrenal gland pheochromocytoma and pituitary gland adenoma or adenocarcinoma in female rats exposed to MBT for 2 years by gavage (NTP, 1988). The incidence data used in the modeling are shown in Table C-8.

Table C-8. Incidences of Selected Neoplastic Lesions in F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Male Rats			
HED, mg/kg-d[b]	**0**	**64.3**	**129**
Mesothelioma	0/50[†]	2/50 (4%)	3/50 (6%)
Adrenal gland pheochromocytoma or malignant pheochromocytoma	18/50[†] (36%)	27/50*[#] (54%)	24/49*[#] (49%)
Preputial gland adenoma or carcinoma	1/50[†] (2%)	6/50* (12%)	5/50* (10%)
Subcutaneous tissue fibroma, neurofibroma, sarcoma, or fibrosarcoma	3/50[†] (6%)	6/50 (12%)	7/50* (14%)
Female Rats			
HED, mg/kg-d[b]	**0**	**32.2**	**64.3**
Pituitary gland adenoma or adenocarcinoma	16/49[†] (33%)	24/50 (48%)	25/50*[#] (50%)
Adrenal gland, pheochromocytoma	1/50[†] (2%)	5/50 (10%)	6/50* (12%)

[a]NTP (1988).
[b]Gavage doses were adjusted for continuous exposure by multiplying the administered gavage dose by (5/7) days/week and converted into HEDs using $BW^{3/4}$ scaling.
*Significantly different from concurrent control at $p < 0.05$ based on life table test performed by the study authors.
[#]Significantly different from concurrent control at $p < 0.05$ based on incidental tumor test performed by the study authors.
[†]Significant ($p < 0.05$) dose-related trend by life table or incidental tumor analysis or both.

BW = body weight; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

MODEL-FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The model-fitting procedure for dichotomous cancer incidence is as follows. The Multistage cancer model in EPA's BMDS, Version 2.5 is fit to the incidence data using the extra risk option. The Multistage cancer model is run for all polynomial degrees up to $n - 1$ (where n is the number of dose groups including control). An adequate model fit is judged by three criteria: (1) goodness-of-fit p-value ($p < 0.1$), (2) visual inspection of the dose-response curve, and (3) scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the benchmark dose lower confidence limit (BMDL) for the model with the lowest AIC is selected as POD when BMDL values were within a factor of 2−3. When BMDL values from models providing adequate fit varied more than two or threefold, the lowest BMDL was selected as a potential POD. In accordance with U.S. EPA (2012b) guidance, benchmark dose (BMD) and BMDL values associated with an extra risk of 10% are calculated.

Model Predictions for Mesothelioma in Male Rats

The procedure outlined above was applied to the data for incidence of mesothelioma in male rats (see Table C-8). The software converged on the 1-degree model, which provided adequate fit ($p > 0.05$); thus, it was selected as the best-fitting model (see Table C-9). The BMD_{10} (HED) and $BMDL_{10}$ (HED) values from this model were 198 and 103 mg/kg-day, respectively. Figure C-2 shows the model fit to the data.

Table C-9. Modeling Results for Mesothelioma in Male F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals	AIC	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)
Multistage cancer (1-degree)[c,d]	2	0.09	0.9546	−0.177	41.5816	197.752	102.662
Multistage cancer (2-degree)[c]	2	0.09	0.9546	−0.177	41.5816	197.752	102.662
Multistage cancer (3-degree)[c]	2	0.09	0.9546	−0.177	41.5816	197.752	102.662

[a]NTP (1988).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. All models provided adequate fit to the data.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degree(s) of freedom; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

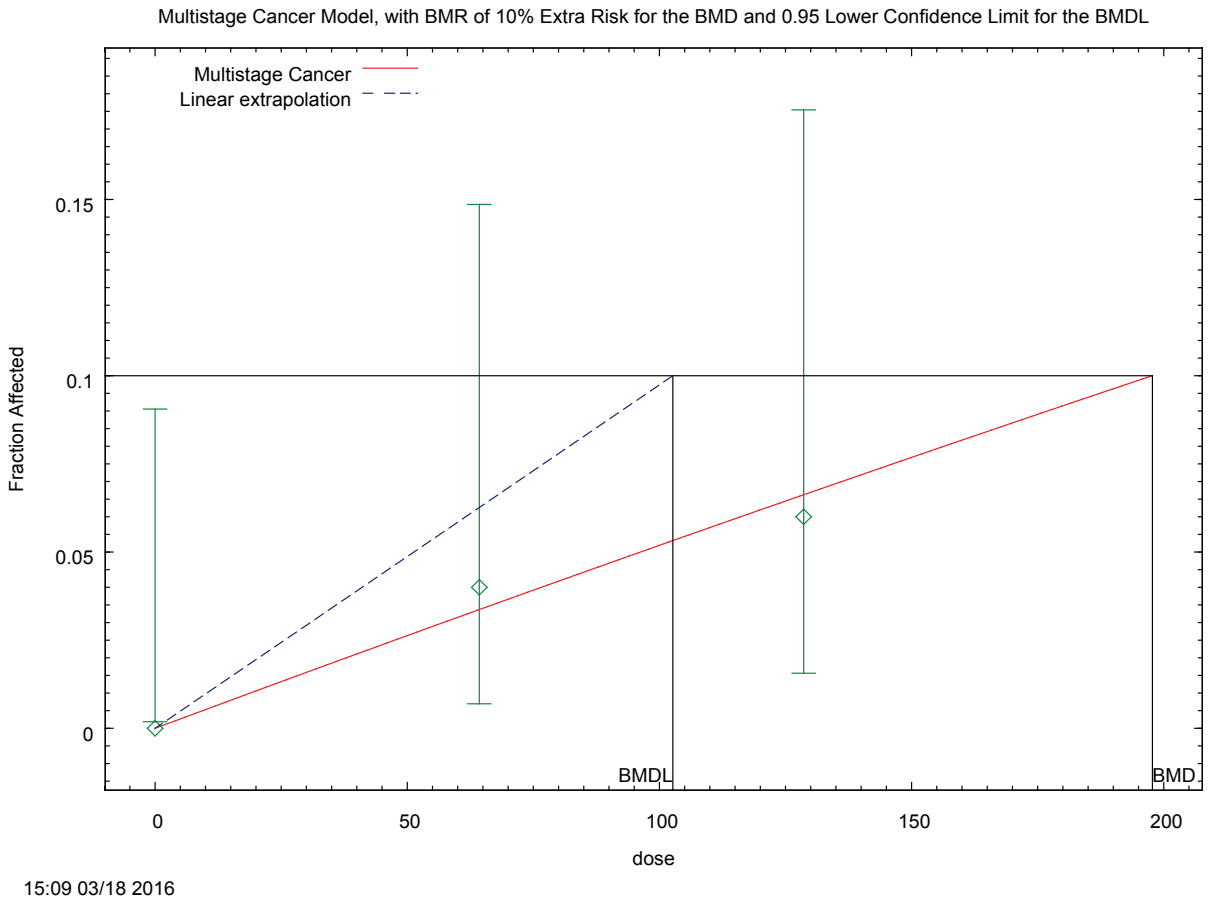

Figure C-2. 1-Degree Multistage Cancer Model for Mesothelioma in Male Rats Administered MBT via Gavage for 104 Weeks (NTP, 1988)

Text Output for Multistage Cancer Model for Mesothelioma in Male Rats Administered MBT via Gavage for 103 Weeks (NTP, 1988)

```
 ======================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS2601/Data/msc_Dichotomous_Opt.(d)
        Gnuplot Plotting File:  C:/Users/bowens/BMDS2601/Data/msc_Dichotomous_Opt.plt
                                          Fri Mar 18 15:20:11 2016
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
```

```
 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0032923
                        Beta(1) =  0.000481135


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
        Beta(1)      0.000532791      0.000238304        6.57237e-005
0.000999858

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -19.7456         3
   Fitted model        -19.7908         1     0.0904085      2          0.9558
  Reduced model        -21.9217         1       4.35226      2          0.1135

           AIC:         41.5816


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      50.000        0.000
   64.2860     0.0337         1.684     2.000      50.000        0.248
  128.6000     0.0662         3.311     3.000      50.000       -0.177
```

```
 Chi^2 = 0.09      d.f. = 2        P-value = 0.9546


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        197.752

            BMDL =        102.662

            BMDU =        740.797

Taken together, (102.662, 740.797) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =   0.000974069
```

Model Predictions for Adrenal Gland Pheochromocytoma or Malignant Pheochromocytoma in Male Rats

The procedure outlined above was applied to the data for incidence of adrenal gland pheochromocytoma or malignant pheochromocytoma in male rats (see Table C-8). The software converged on the 1-degree model, which provided adequate fit ($p > 0.05$); thus, it was selected as the best-fitting model (see Table C-10). The BMD_{10} (HED) and $BMDL_{10}$ (HED) values from this model were 51.1 and 23.0 mg/kg-day, respectively. Figure C-3 shows the model fit to the data.

Table C-10. Modeling Results for Adrenal Gland Pheochromocytoma or Malignant Pheochromocytoma in Male F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals	AIC	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)
Multistage cancer (1-degree)[c,d]	1	1.65	0.1991	1.043	207.891	51.1241	23.0143
Multistage cancer (2-degree)[c]	1	1.65	0.1991	1.043	207.891	51.1241	23.0143
Multistage cancer (3-degree)[c]	1	1.65	0.1991	1.043	207.891	51.1241	23.0143

[a]NTP (1988).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. All models provided adequate fit to the data.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degree(s) of freedom; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

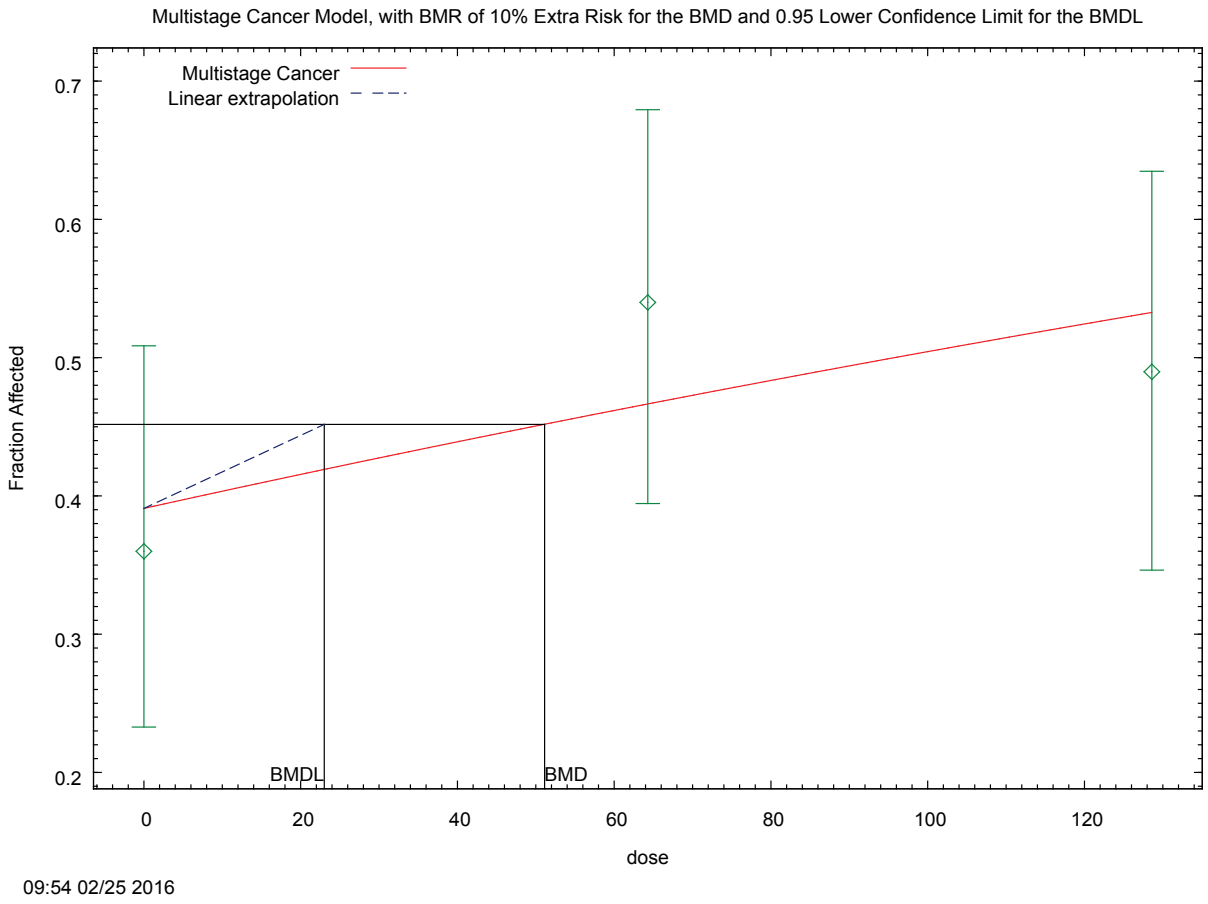

Figure C-3. 1-Degree Multistage Cancer Model for Adrenal Gland Pheochromocytoma in Male Rats Administered MBT via Gavage for 104 Weeks (NTP, 1988)

Text Output for Multistage Cancer Model for Adrenal Gland Pheochromocytoma or Malignant Pheochromocytoma in Male Rats Administered MBT via Gavage for 103 Weeks (NTP, 1988)

```
================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS2601/Data/msc_Dichotomous2_Opt.(d)
        Gnuplot Plotting File:
C:/Users/bowens/BMDS2601/Data/msc_Dichotomous2_Opt.plt
                                          Thu Feb 25 09:54:35 2016
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]
```

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.404656
Beta(1) = 0.00176225

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.73
Beta(1)	-0.73	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.390834	0.0666806	0.260142	0.521525
Beta(1)	0.00206088	0.00151448	-0.000907443	0.00502919

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-101.122	3			
Fitted model	-101.946	2	1.64683	1	0.1994
Reduced model	-102.873	1	3.50083	2	0.1737

AIC: 207.891

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3908	19.542	18.000	50.000	-0.447
64.2860	0.4664	23.321	27.000	50.000	1.043
128.6000	0.5327	26.100	24.000	49.000	-0.601

```
 Chi^2 = 1.65      d.f. = 1        P-value = 0.1991


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        51.1241

            BMDL =        23.0143

            BMDU =   6.54617e+007

Taken together, (23.0143, 6.54617e+007) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =    0.00434512
```

Model Predictions for Preputial Gland Adenoma or Carcinoma in Male Rats

The procedure outlined above was applied to the data for incidence of preputial gland adenoma or carcinoma in male rats (see Table C-8). The software converged on the 1-degree model, which provided adequate fit ($p > 0.05$); thus, it was selected as the best-fitting model (see Table C-11). The BMD_{10} (HED) and $BMDL_{10}$ (HED) values from this model were 118 and 62.5 mg/kg-day, respectively. Figure C-4 shows the model fit to the data.

Table C-11. Modeling Results for Preputial Gland Adenoma or Carcinoma in Male F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals	AIC	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)
Multistage cancer (1-degree)[c,d]	1	1.57	0.2107	−0.67	84.4896	117.939	62.4815
Multistage cancer (2-degree)[c]	1	1.57	0.2107	−0.67	84.4896	117.939	62.4815
Multistage cancer (3-degree)[c]	1	1.57	0.2107	−0.67	84.4896	117.939	62.4815

[a]NTP (1988).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. All models provided adequate fit to the data.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degree(s) of freedom; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

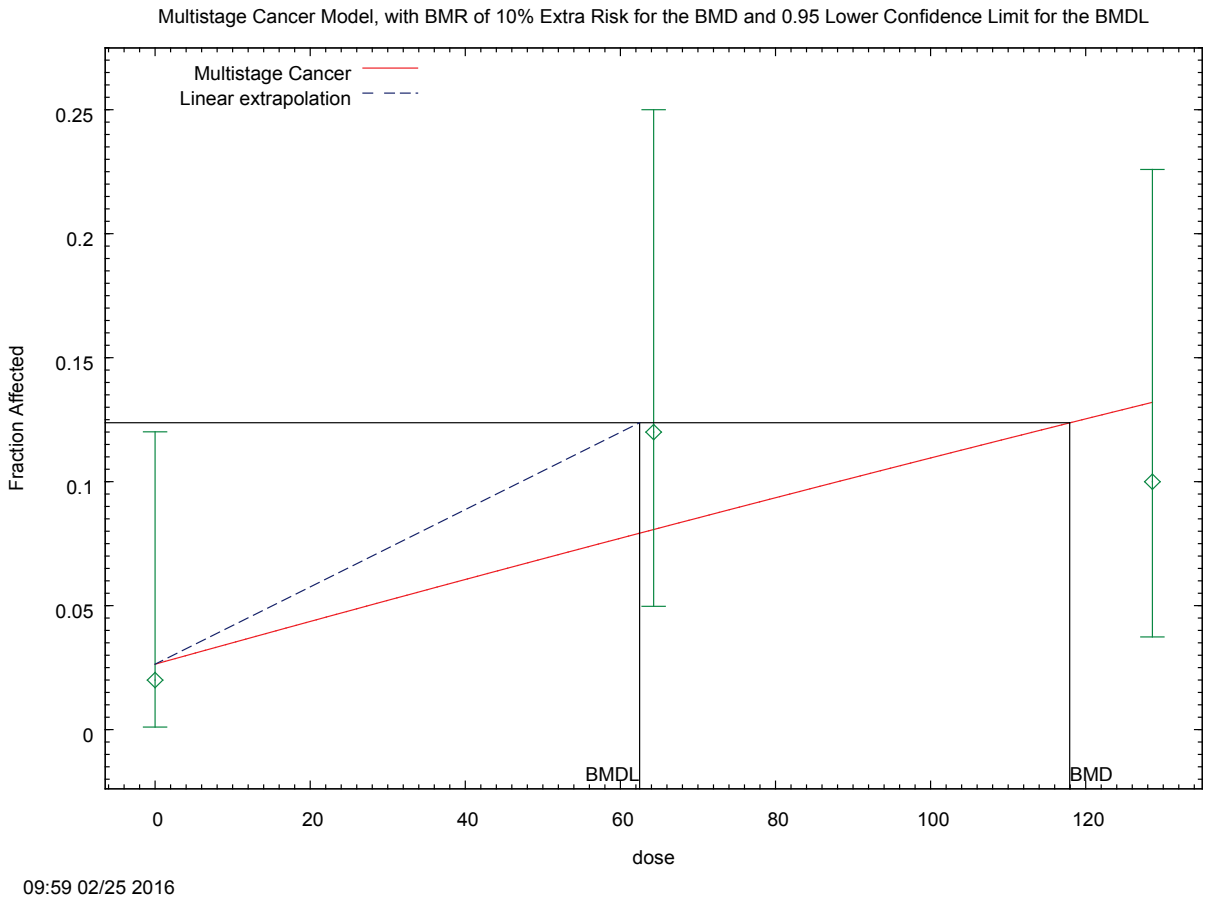

Figure C-4. 1-Degree Multistage Cancer Model for Preputial Gland Adenoma or Carcinoma in Male Rats Administered MBT via Gavage for 104 Weeks (NTP, 1988)

Text Output for Multistage Cancer Model for Preputial Gland Adenoma or Carcinoma in Male Rats Administered MBT via Gavage for 103 Weeks (NTP, 1988)

```
 ======================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS2601/Data/msc_Dichotomous3_Opt.(d)
        Gnuplot Plotting File:
C:/Users/bowens/BMDS2601/Data/msc_Dichotomous3_Opt.plt
                                                Thu Feb 25 09:59:43 2016
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = Effect
  Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =     0.041029
                        Beta(1) =  0.000662118


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.63

   Beta(1)        -0.63            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0264206         0.025062          -0.0227001
0.0755413
        Beta(1)       0.00089335      0.000465605        -1.9218e-005
0.00180592


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -39.5024         3
   Fitted model        -40.2448         2       1.48486      1           0.223
  Reduced model        -41.8154         1        4.6261      2         0.09896

           AIC:         84.4896


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0264         1.321     1.000      50.000       -0.283
   64.2860     0.0808         4.038     6.000      50.000        1.018
  128.6000     0.1321         6.604     5.000      50.000       -0.670
```

```
 Chi^2 = 1.57      d.f. = 1        P-value = 0.2107


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        117.939

            BMDL =        62.4815

            BMDU =        1507.44

Taken together, (62.4815, 1507.44) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =    0.00160047
```

Model Predictions for Subcutaneous Tissue Fibroma, Neurofibroma, Sarcoma, or Fibrosarcoma in Male Rats

The procedure outlined above was applied to the data for incidence of subcutaneous tissue fibroma, neurofibroma, sarcoma, or fibrosarcoma in male rats (see Table C-8). The software converged on the 1-degree model, which provided adequate fit ($p > 0.05$); thus, it was selected as the best-fitting model (see Table C-12). The BMD_{10} (HED) and $BMDL_{10}$ (HED) values from this model were 142 and 63.7 mg/kg-day, respectively. Figure C-5 shows the model fit to the data.

Table C-12. Modeling Results for Subcutaneous Tissue Fibroma, Neurofibroma, Sarcoma, or Fibrosarcoma in Male F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals	AIC	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)
Multistage cancer (1-degree)[c,d]	1	0.13	0.7217	−0.175	104.01	142.3	63.7278
Multistage cancer (2-degree)[c]	1	0.13	0.7217	−0.175	104.01	142.3	63.7278
Multistage cancer (3-degree)[c]	1	0.13	0.7217	−0.175	104.01	142.3	63.7278

[a]NTP (1988).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. All models provided adequate fit to the data.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degree(s) of freedom; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

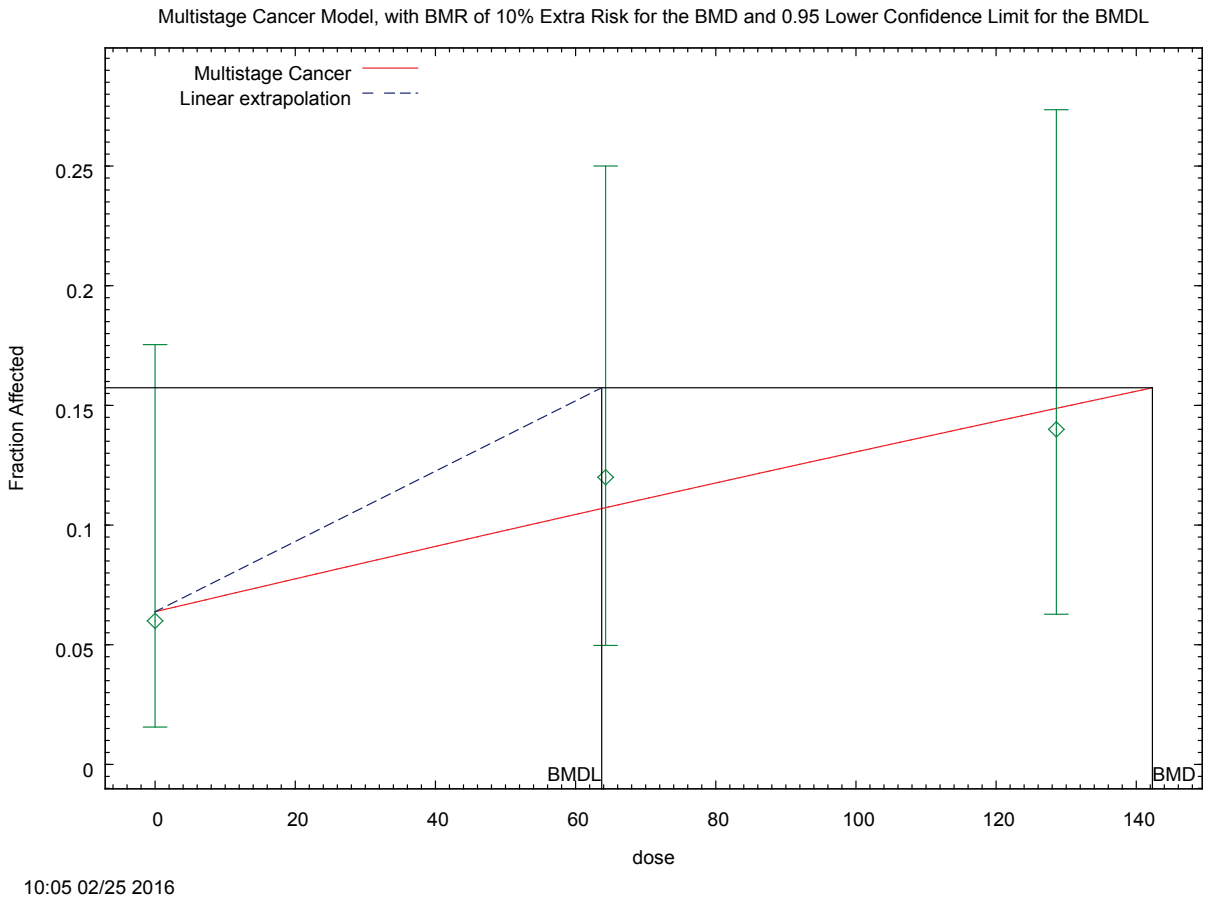

Figure C-5. 1-Degree Multistage Cancer Model for Subcutaneous Tissue Fibroma, Neurofibroma, Sarcoma, or Fibrosarcoma in Male Rats Administered MBT via Gavage for 103 Weeks (NTP, 1988)

Text Output for Multistage Cancer Model for Subcutaneous Tissue Fibroma, Neurofibroma, Sarcoma, or Fibrosarcoma in Male Rats Administered MBT via Gavage for 103 Weeks (NTP, 1988)

```
 ================================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS2601/Data/msc_Dichotomous4_Opt.(d)
        Gnuplot Plotting File:
C:/Users/bowens/BMDS2601/Data/msc_Dichotomous4_Opt.plt
                                                Thu Feb 25 10:05:38 2016
 ================================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]
```

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0667121
Beta(1) = 0.000691636

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.68
Beta(1)	-0.68	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0637767	0.0337003	-0.00227464	0.129828
Beta(1)	0.000740409	0.000538242	-0.000314526	0.00179534

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-49.9428	3			
Fitted model	-50.0052	2	0.124811	1	0.7239
Reduced model	-50.9233	1	1.96108	2	0.3751

AIC: 104.01

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0638	3.189	3.000	50.000	-0.109
64.2860	0.1073	5.365	6.000	50.000	0.290

```
  128.6000     0.1488         7.440     7.000      50.000       -0.175

 Chi^2 = 0.13      d.f. = 1        P-value = 0.7217


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =          142.3

            BMDL =        63.7278

            BMDU =   2.10827e+008

Taken together, (63.7278, 2.10827e+008) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =    0.00156917
```

Model Predictions for MS_Combo-Multiple Tumor Model for All Tumor Types in Male Rats

MS_Combo-multiple tumor BMD modeling was used to combine tumor incidence data for mesothelioma, adrenal gland pheochromocytoma or malignant pheochromocytoma, preputial gland adenoma or carcinoma, and subcutaneous tissue fibroma, neurofibroma, sarcoma, or fibrosarcoma in male rats. For each tumor type, the best-fitting Multistage model (i.e., the degree of polynomial setting) was maintained in the MS_Combo model run. The calculated combined tumor $BMDL_{10}$ (HED) based on the MS_Combo model is 15.0 mg/kg-day.

Text Output for MS_COMBO Multiple Tumor Model for Combined Tumors in Male Rats

```
=====================================================================
         MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
         Input Data File: C:\Users\bowens\BMDS2601\Data\multi_test.(d)
         Gnuplot Plotting File:  C:\Users\bowens\BMDS2601\Data\multi_test.plt
                                              Fri Mar 18 15:18:23 2016
 =====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = Dichotomous.dax
```

```
 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0032923
                        Beta(1) =  0.000481135


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0            *                *                   *
        Beta(1)      0.000532791            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -19.7456         3
   Fitted model         -19.7908         1     0.0904085      2          0.9558
  Reduced model         -21.9217         1       4.35226      2          0.1135

           AIC:          41.5816

 Log-likelihood Constant             16.99398096819764


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size          Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      50.000          0.000
   64.2860     0.0337         1.684     2.000      50.000          0.248
  128.6000     0.0662         3.311     3.000      50.000         -0.177
```

```
 Chi^2 = 0.09      d.f. = 2        P-value = 0.9546


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        197.752

            BMDL =        102.662

            BMDU =        740.797

Taken together, (102.662, 740.797) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =   0.000974069



 ======================================================================
        MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
        Input Data File: C:\Users\bowens\BMDS2601\Data\multi_test.(d)
        Gnuplot Plotting File:  C:\Users\bowens\BMDS2601\Data\multi_test.plt
                                              Fri Mar 18 15:18:23 2016
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = Dichotomous2.dax

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.404656
                        Beta(1) =   0.00176225
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.76
Beta(1)	-0.76	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.390834	*	*	*
Beta(1)	0.00206088	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-101.122	3			
Fitted model	-101.946	2	1.64683	1	0.1994
Reduced model	-102.873	1	3.50083	2	0.1737

AIC: 207.891

Log-likelihood Constant 94.615324369879303

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3908	19.542	18.000	50.000	-0.447
64.2860	0.4664	23.321	27.000	50.000	1.043
128.6000	0.5327	26.100	24.000	49.000	-0.601

Chi^2 = 1.65 d.f. = 1 P-value = 0.1991

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 51.1241

BMDL = 23.0143

BMDU = 6.54617e+007

Taken together, (23.0143, 6.54617e+007) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00434512

```
 ======================================================================
        MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
        Input Data File: C:\Users\bowens\BMDS2601\Data\multi_test.(d)
        Gnuplot Plotting File:  C:\Users\bowens\BMDS2601\Data\multi_test.plt
                                            Fri Mar 18 15:18:23 2016
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = Dichotomous3.dax

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.041029
                        Beta(1) =  0.000662118


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.81

   Beta(1)        -0.81            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0264206            *               *                 *
        Beta(1)       0.00089335            *               *                 *

* - Indicates that this value is not calculated.
```

```
                    Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -39.5024         3
   Fitted model        -40.2448         2       1.48486      1          0.223
  Reduced model        -41.8154         1        4.6261      2        0.09896

           AIC:         84.4896

 Log-likelihood Constant             35.059609165698149


                               Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0264         1.321     1.000      50.000       -0.283
   64.2860     0.0808         4.038     6.000      50.000        1.018
  128.6000     0.1321         6.604     5.000      50.000       -0.670

 Chi^2 = 1.57      d.f. = 1        P-value = 0.2107


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        117.939

            BMDL =        62.4815

            BMDU =        1507.44

Taken together, (62.4815, 1507.44) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00160047



 ======================================================================
        MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
        Input Data File: C:\Users\bowens\BMDS2601\Data\multi_test.(d)
        Gnuplot Plotting File:  C:\Users\bowens\BMDS2601\Data\multi_test.plt
                                           Fri Mar 18 15:18:23 2016
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
   Dependent variable = Effect
   Independent variable = Dose
   Data file name = Dichotomous4.dax

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0667121
                        Beta(1) =  0.000691636


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.77

   Beta(1)        -0.77            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0637767            *               *                   *
        Beta(1)      0.000740409            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -49.9428         3
   Fitted model        -50.0052         2      0.124811      1          0.7239
  Reduced model        -50.9233         1       1.96108      2          0.3751

           AIC:          104.01

 Log-likelihood Constant            44.884053510893828


                               Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size          Residual
  ---------------------------------------------------------------------------
    0.0000     0.0638         3.189     3.000      50.000         -0.109
   64.2860     0.1073         5.365     6.000      50.000          0.290
  128.6000     0.1488         7.440     7.000      50.000         -0.175
```

```
 Chi^2 = 0.13      d.f. = 1        P-value = 0.7217


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =          142.3

            BMDL =        63.7278

            BMDU =   1.71842e+008

Taken together, (63.7278, 1.71842e+008) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00156917


**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                     -211.98629808735791

  Combined Log-likelihood Constant             191.55296801466895


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        24.9231

            BMDL =        14.9871

Multistage Cancer Slope Factor =    0.00667239
```

Model Predictions for Adrenal Gland Pheochromocytoma in Female Rats

The procedure outlined above was applied to the data for incidence of adrenal gland pheochromocytoma in female rats (see Table C-8). Table C-13 summarizes the BMD modeling results. Both the 1- and 2-degree Multistage cancer models converged on the same model, yielding a BMD_{10} (HED) and $BMDL_{10}$ (HED) of 55.0 and 30.5 mg/kg-day, respectively (see Figure C-6 and the BMD text output for details).

Table C-13. Modeling Results for Increased Incidence of Adrenal Gland Pheochromocytoma in Female F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals	AIC	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)
Multistage cancer (1-degree)[c,d]	1	0.36	0.5478	−0.327	83.3553	55.0149	30.5228
Multistage cancer (2-degree)[c]	1	0.36	0.5478	−0.327	83.3553	55.0149	30.5228

[a]NTP (1988).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. Both models provided adequate fit to the data. The Multistage cancer (2-degree) converged upon the Multistage cancer (1-degree), so the Multistage cancer (1-degree) was selected.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degree(s) of freedom; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

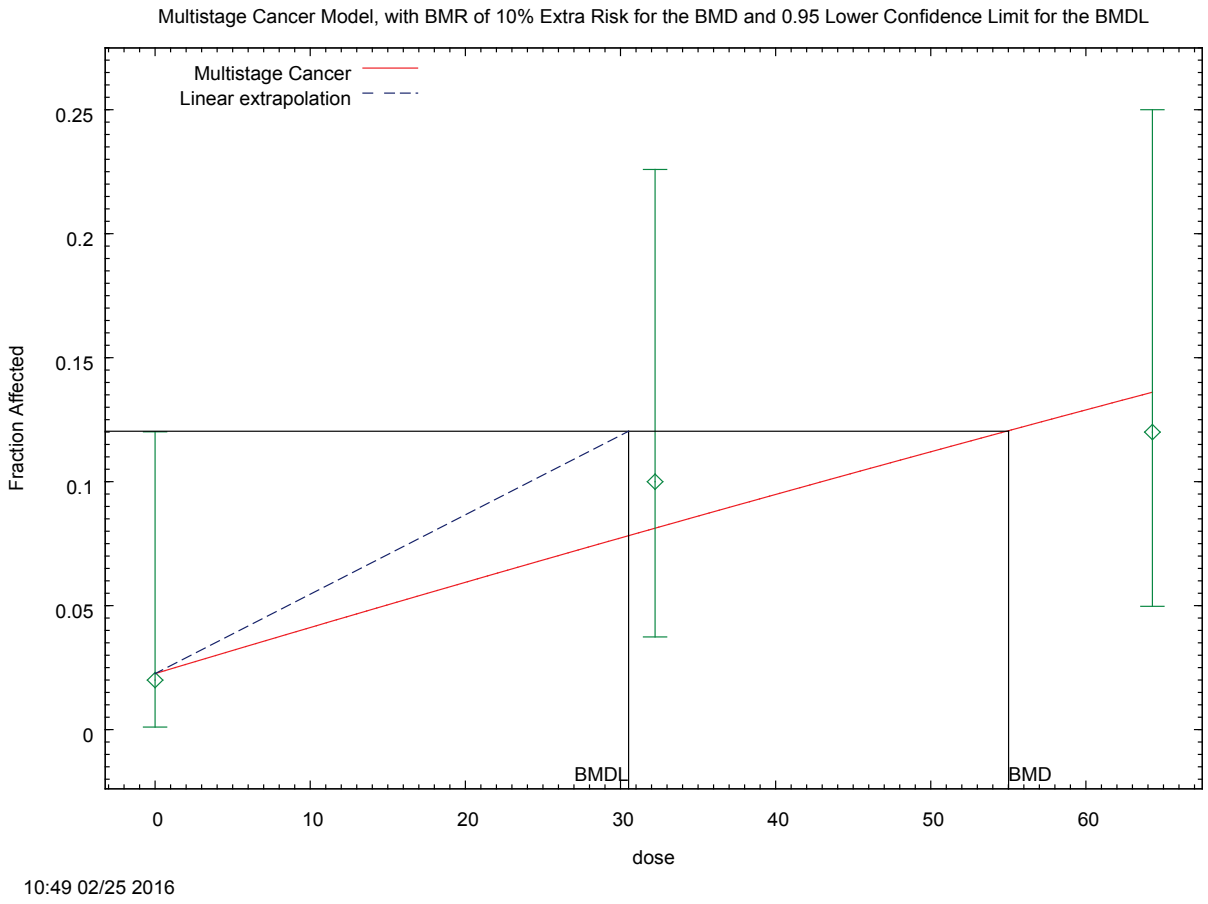

Figure C-6. 1-Degree Multistage Cancer Model for Adrenal Gland Pheochromocytoma in Female Rats Administered MBT via Gavage for 104 Weeks (NTP, 1988)

Text Output for 1-Degree Multistage Cancer for Adrenal Gland Pheochromocytoma in Female Rats Administered MBT via Gavage for 104 Weeks (NTP, 1988)

```
 ======================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS2601/Data/msc_Dichotomous cancer_Opt.(d)
        Gnuplot Plotting File:  C:/Users/bowens/BMDS2601/Data/msc_Dichotomous
cancer_Opt.plt
                                                Thu Feb 25 10:49:22 2016
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive
```

```
  Dependent variable = Effect
  Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0301108
                        Beta(1) =   0.00167512


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.55

   Beta(1)        -0.55            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0226243        0.0217662          -0.0200367
0.0652852
        Beta(1)       0.00191513      0.000871465          0.000207087
0.00362317


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -39.5024         3
   Fitted model        -39.6776         2      0.350555      1          0.5538
  Reduced model        -41.8154         1        4.6261      2         0.09896

           AIC:         83.3553


                               Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0226         1.131     1.000      50.000       -0.125
   32.2300     0.0811         4.056     5.000      50.000        0.489
   64.2860     0.1358         6.792     6.000      50.000       -0.327
```

```
 Chi^2 = 0.36      d.f. = 1        P-value = 0.5478


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        55.0149

            BMDL =        30.5228

            BMDU =        248.014

Taken together, (30.5228, 248.014) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =    0.00327624
```

Model Predictions for Pituitary Gland Adenoma or Adenocarcinoma in Female Rats

The procedure outlined above was applied to the data for incidence of pituitary gland adenoma or adenocarcinoma in female rats (see Table C-8). Table C-14 summarizes the BMD modeling results. Both the 1- and 2-degree Multistage cancer models converged on the same model, yielding a BMD_{10} (HED) and $BMDL_{10}$ (HED) of 21.0 and 10.9 mg/kg-day (see Figure C-7 and the BMD text output for details).

Table C-14. Modeling Results for Increased Incidence of Pituitary Gland Adenoma or Adenocarcinoma in Female F344/N Rats Exposed to MBT by Gavage for 103 Weeks[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals	AIC	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)
Multistage cancer (1-degree)[c,d]	1	0.48	0.4873	0.564	204.937	20.9922	10.8656
Multistage cancer (2-degree)[c]	1	0.48	0.4873	0.564	204.937	20.9922	10.8656

[a]NTP (1988).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. Both models provided adequate fit to the data. The Multistage cancer (2-degree) converged upon the Multistage cancer (1-degree), so the Multistage cancer (1-degree) was selected.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degree(s) of freedom; HED = human equivalent dose; MBT = 2-mercaptobenzothiazole.

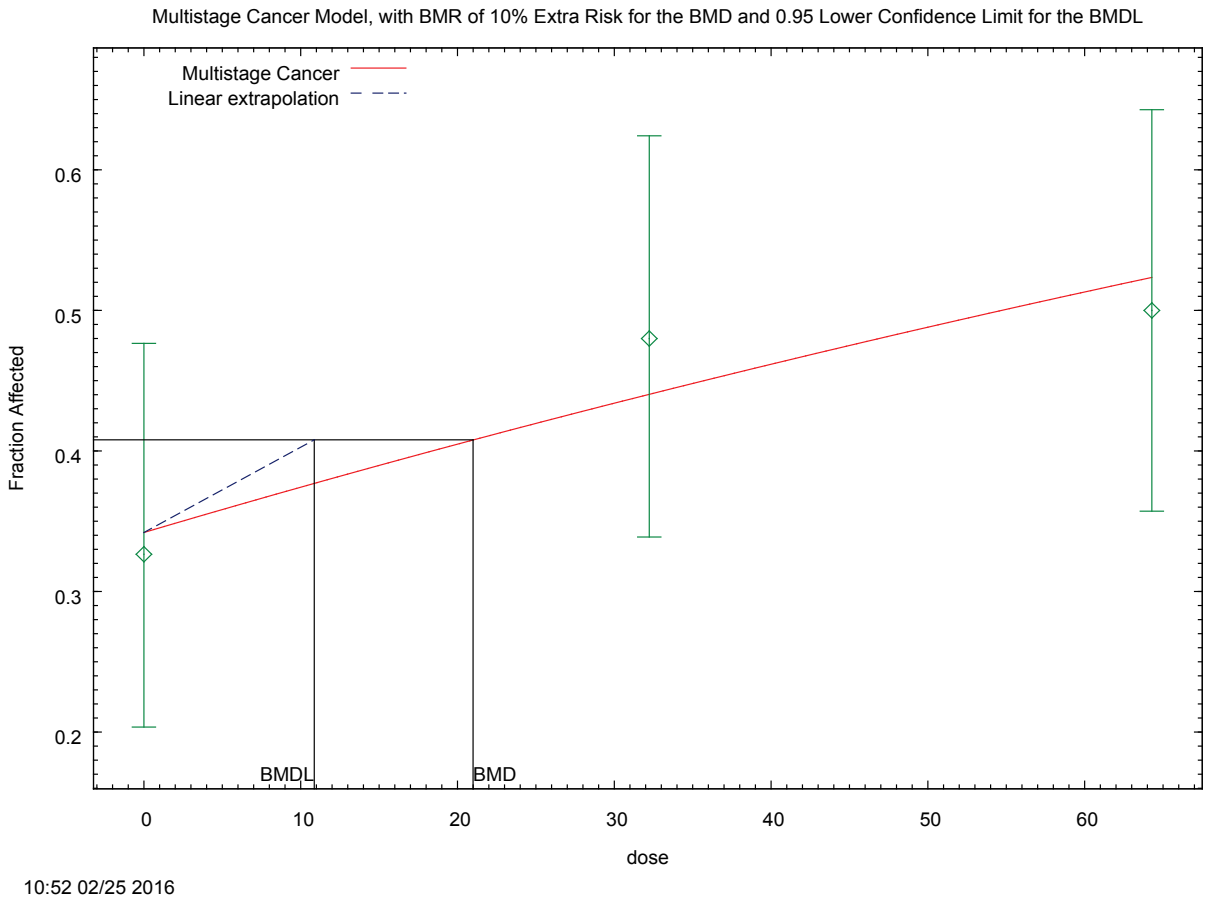

Figure C-7. 1-Degree Multistage Cancer Model for Pituitary Gland Adenoma or Adenocarcinoma in Female Rats Administered MBT via Gavage for 104 Weeks (NTP, 1988)

Text Output for 1-Degree Multistage Cancer Model for Pituitary Gland Adenoma or Adenocarcinoma in Female Rats Administered MBT via Gavage for 104 Weeks (NTP, 1988)

```
 ======================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS2601/Data/msc_Dichotomous_Opt.(d)
        Gnuplot Plotting File:  C:/Users/bowens/BMDS2601/Data/msc_Dichotomous_Opt.plt
                                          Thu Feb 25 10:52:08 2016
 ======================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]
```

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.35056
Beta(1) = 0.00463603

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.7
Beta(1)	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.342175	0.065075	0.21463	0.469719
Beta(1)	0.00501904	0.00280774	-0.000484026	0.0105221

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-100.228	3			
Fitted model	-100.468	2	0.481126	1	0.4879
Reduced model	-102.064	1	3.67297	2	0.1594

AIC: 204.937

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3422	16.767	16.000	49.000	-0.231
32.2300	0.4404	22.021	24.000	50.000	0.564
64.2860	0.5236	26.179	25.000	50.000	-0.334

```
 Chi^2 = 0.48      d.f. = 1        P-value = 0.4873


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        20.9922

            BMDL =        10.8656

            BMDU =        263.023

Taken together, (10.8656, 263.023) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =    0.00920333
```

Model Predictions for MS_Combo-Multiple Tumor Model for All Tumor Types in Female Rats

MS_Combo-multiple tumor BMD modeling was used to combine tumor incidence data for adrenal gland pheochromocytoma and pituitary gland adenoma or adenocarcinoma in female rats. For each tumor type, the best-fitting Multistage model (i.e., the degree of polynomial setting) was maintained in the MS_Combo model run. The calculated combined tumor $BMDL_{10}$ (HED) based on the MS_Combo model is 8.91 mg/kg-day. This $BMDL_{10}$ (HED) is used as the POD to derive the provisional oral slope factor (p-OSF).

Text Output for MS_COMBO Multiple Tumor Model for Combined Tumors in Female Rats

```
======================================================================
         MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
         Input Data File: C:\Users\bowens\BMDS2601\Data\multi_test.(d)
         Gnuplot Plotting File:  C:\Users\bowens\BMDS2601\Data\multi_test.plt
                                              Thu Feb 25 11:11:37 2016
 ======================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = Dichotomous.dax
```

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.35056
Beta(1) = 0.00463603

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.75
Beta(1)	-0.75	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.342175	*	*	*
Beta(1)	0.00501904	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-100.228	3			
Fitted model	-100.468	2	0.481126	1	0.4879
Reduced model	-102.064	1	3.67297	2	0.1594

AIC: 204.937

Log-likelihood Constant 93.741309121153364

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3422	16.767	16.000	49.000	-0.231
32.2300	0.4404	22.021	24.000	50.000	0.564
64.2860	0.5236	26.179	25.000	50.000	-0.334

Chi^2 = 0.48 d.f. = 1 P-value = 0.4873

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        20.9922

            BMDL =        10.8656

            BMDU =        263.023

Taken together, (10.8656, 263.023) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00920333



 ======================================================================
        MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
        Input Data File: C:\Users\bowens\BMDS2601\Data\multi_test.(d)
        Gnuplot Plotting File:  C:\Users\bowens\BMDS2601\Data\multi_test.plt
                                              Thu Feb 25 11:11:37 2016
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = Dichotomouscancer.dax

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0301108
                        Beta(1) =   0.00167512


           Asymptotic Correlation Matrix of Parameter Estimates
```

	Background	Beta(1)
Background	1	-0.78
Beta(1)	-0.78	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0226243	*	*	*
Beta(1)	0.00191513	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-39.5024	3			
Fitted model	-39.6776	2	0.350555	1	0.5538
Reduced model	-41.8154	1	4.6261	2	0.09896

AIC: 83.3553

Log-likelihood Constant 35.059609165698149

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0226	1.131	1.000	50.000	-0.125
32.2300	0.0811	4.056	5.000	50.000	0.489
64.2860	0.1358	6.792	6.000	50.000	-0.327

Chi^2 = 0.36 d.f. = 1 P-value = 0.5478

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 55.0149

BMDL = 30.5228

BMDU = 248.014

Taken together, (30.5228, 248.014) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00327624

```
**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                    -140.14592704037494

  Combined Log-likelihood Constant            128.80091828685153


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        15.1944

            BMDL =         8.9079

Multistage Cancer Slope Factor =      0.011226
```

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