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Provisional Peer-Reviewed Toxicity Values for

Butylated Hydroxytoluene (BHT)
(CASRN 128-37-0)

Superfund Health Risk Technical Support Center
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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR BUTYLATED HYDROXYTOLUENE (CASRN 128-37-0)

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 (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.

QUESTIONS REGARDING PPRTVS

Questions regarding the contents and appropriate use 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

Butylated hydroxytoluene (BHT) is a phenol that is a colorless solid at room temperature (OECD SIDS, 2002). The empirical formula for BHT is $C_{15}H_{24}O$ (see Figure 1). A table of physicochemical properties is provided below (see Table 1).

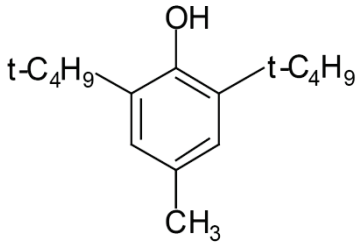

Figure 1. Butylated Hydroxytoluene Structure

Table 1. Physicochemical Properties of Butylated Hydroxytoluene (CASRN 128-37-0)[a]

Property (unit)	Value
Boiling point (ºC)	265
Melting point (ºC)	70
Density (g/cm^3)	1.03
Vapor pressure (Pa at 20ºC)	1.1
pH (unitless)	Not available
Solubility in water (mg/L at 20−25ºC)	0.6−1.1
Relative vapor density (air = 1)	Not available
Molecular weight (g/mol)	220.35

[a]OECD SIDS (2002).

No Reference Dose (RfD), Reference Concentration (RfC), or cancer assessment for BHT is included on the IRIS database (U.S. EPA, 2010). The Drinking Water Standards and Health Advisories List does not report values for BHT (U.S. EPA, 2006). The Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 2010) does not report any RfD or RfC values. The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994a) does not provide a Health and Environmental Effects Profile (HEEP) for BHT. The toxicity of BHT has not been reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2008). The World Health Organization (WHO, 1996) has reviewed the toxicity of BHT and reports an acceptable daily intake (ADI) of 0−0.3 mg/kg-body weight (bw). The California Environmental Protection Agency (CalEPA, 2008) has not derived toxicity values for exposure to BHT. The American Conference of Governmental Industrial Hygienists (ACGIH, 2008) has derived a threshold limit value (TLV; 8-hour time-weighted average) of 2 mg/m^3. The National Institute of Occupational Safety and Health (NIOSH, 2011) has derived a recommended exposure limit (REL; 10-hour time-weighted average) of 10 mg/m^3. No occupational exposure

limits are currently available from the Occupational Safety and Health Administration (OSHA, 2006).

The HEAST (U.S. EPA, 2010) has not reported an EPA (1986) cancer weight-of-evidence classification for BHT. The ACGIH (2008) classifies BHT as "*Not Classifiable as a Human Carcinogen*." The International Agency for Research on Cancer (IARC, 2010) classifies BHT as having "limited evidence" to determine carcinogenicity to animals and states that "no evaluation could be made of the carcinogenicity of butylated hydroxytoluene in humans" (IARC, 1986). BHT is not included in the *12th Report on Carcinogens* (NTP, 2011). CalEPA (2008) has not prepared a quantitative estimate of carcinogenic potential for BHT.

BHT has an extensive literature database due to its wide use (3529 references). In order to develop a thorough list of relevant studies, the following methodology was employed. Literature searches, using the Chemical Abstracts Service (CAS) registry number, were conducted on sources published from 1900 through July 2011, for studies potentially relevant to the derivation of provisional toxicity values for BHT, CAS No. 128-37-0. This search resulted in the identification of 3529 references. Comprehensive toxicity reviews of BHT by OECD SIDS (2002) and WHO (1996) revealed 19 studies pertinent to PPRTV toxicity value derivation. A secondary literature search of studies after 2002 was conducted, and all relevant literature was requested and incorporated into the PPRTV document (39 studies). Due to the equivocal nature of the literature on the carcinogenic potential of BHT, a focused genotoxicity search was conducted to support the carcinogenicity assessment. Studies cited by Williams et al. (1999) in a comprehensive genotoxicity review were requested for evaluation and review (15 studies). An additional search for genotoxicity data published after 1999 was conducted, and all relevant studies were retrieved for evaluation and reviewed (9 studies). Any study mentioned in any of the retrieved articles that seemed relevant to the derivation of a PPRTV value was requested and reviewed. In total, 82 documents were retrieved and reviewed for this PPRTV document.

Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI, and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. Given the large quantity of data available for BHT, a selection of all appropriate well-conducted relevant studies for incorporation into the PPRTV document were reviewed and incorporated into the PPRTV document on BHT. The following databases outside of HERO were searched for relevant health information: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides an overview of the database for BHT and includes all potentially relevant repeated short-term-, subchronic-, and chronic-duration studies. The phrase, "statistical significance" used throughout the document, indicates a *p*-value of <0.05.

Table 2. Summary of Potentially Relevant Data for Butylated Hydroxytoluene (CASRN 128-37-0)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Human								
			1. Oral (mg/kg-day)[a]					
Acute	0/2 human, oral, single dose	4 and 80 g	Severe epigastric cramping; nausea; vomiting; neurological disorders	N/D	N/D	N/D	Grogan (1986); Shlian and Goldstone (1986)	
Subchronic	None							
Chronic	None							
Developmental and Reproductive	None							
Carcinogenic	2035 men and women, diet, ~6.3 years	351 µg/day on average	No association found between exposure and stomach cancer incidence	N/D	N/D	N/D	Botterweck et al. (2000)	
Other	2/0 patients, dermal bandages, unknown	Unknown	Eczema and skin sensitivity	N/D	N/D	N/D	Dissanayake and Powell (1989)	
Other	1336 men and women, dermal patch test, 2 days, 3 days or 1 week	Unknown	Patch test results: negative	N/D	N/D	N/D	Flyvholm and Menne (1990)	
			2. Inhalation (mg/m^3)[a]					
Subchronic	None							
Chronic	None							
Developmental and Reproductive	None							
Carcinogenic	None							

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Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Animal								
			1. Oral (mg/kg-day)[a]					
Short-term	5/0 Wistar rat, gavage, 7 and 28 days	7 day: 0, 25, 250, 607 28 day: 0, 25, 250, 527	Progressive periportal hepatocyte necrosis, fibrosis, and hyperplasia; increased relative liver weight	250	No fit	607 for 7-day study and 527 for the 28-day study	Powell et al. (1986)	
	5−10/0, albino Wistar rat, diet, 25 days	0, 678, 811	Decreased food consumption and body-weight gains	678	Not run	811	Deichmann et al. (1955a)	
	4 (sex not reported), mongrel dog, diet, 28 days	0, 600, 900, 1371, 2014	No effects observed	2014	Not run	N/D	Deichmann et al. (1955b)	
Subchronic	10/0 Wistar rat, diet, 8 weeks	0, 30, 151, 755, 1132	Increased absolute and relative liver weights; decreased body weight	N/D	Not run	30	Fulton et al. (1980)	
	3 (sex not reported), albino Wistar rat, diet, 90 days	0, 193, 483, 772, 965, 1448	Increased mortality ≥483 mg/kg-day; decreased food consumption	193	Not run	483 (FEL)	Deichmann et al. (1955c)	
	5/5 F344 rat, diet, 7 weeks	Males: 0, 620, 1250, 2500, 5000 Females: 0, 700, 1411, 2822, 5645	Decreased body weight; increased hematopoiesis High dose: 100% mortality	N/D	Not run	620	NCI (1979a)	

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Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Subchronic	5/5 B6C3F$_1$ mouse, diet, 7 weeks	Males: 0, 559, 1118, 2255, 4510, 9019 Females: 0, 605, 1210, 2439, 4878, 9756	Decreased body weight High dose: increased mortality and slight centrilobular cytoplasmic vacuolization of hepatocytes in males	N/D	Not run	559	NCI (1979b)	
Chronic	21/0 Fisher 344 rat, diet, 76 weeks	0, 8, 24, 79, 237, 474	Decreased body weight; increased relative liver weight	79	Not run	237	Williams et al. (1990)	
	27/0 Fisher 344 rat, diet, 110 weeks	0, 947	Decreased body weight; increased mortality in both the control and treated groups after 84 weeks	N/D	Not run	947	Williams et al. (1990)	
	12/0, F344 rat, diet, 36 weeks	0, 700	25% mortality; decreased body weight; increased relative liver and kidney weights	Not identified	Not run	700 (FEL)	Hirose et al. (1993)	
	57/57 Wistar rat, diet, 104 weeks	Males: 0, 184, 736 Females: 0, 210, 842	Increased liver weight; decreased spleen weights seen in females; serum triglyceride and γ-GTP0- levels altered in treated males and in total blood cholesterol in treated females High dose: increased mortality in males after Week 96; decreased body weights in males	N/D	Not run	184	Hirose et al. (1981)	
	15/15 albino Wistar rat, diet, 2 years	Males: 0, 147, 368, 589, 736 Females: 0, 168, 421, 673, 842	Decreased body weight; increased relative brain, lung, kidney and liver weights in females; increased relative brain, liver, kidney, and testes weights in males	N/D	Not run	168	Deichmann et al. (1955d)	

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Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Chronic	40/40 JCL:S-D rat, diet, 3−24 months	0, 2.5, 10, 160	Increased serum potassium and serum cholesterol; decreased transaminase activity High dose: increased mortality at 24 months; increased liver weight; altered liver and kidney morphologies	N/D	Not run	N/D	Tokyo Metropolitan Research Laboratory of Public Health (1992a); data tables are barely legible and, therefore, the qualitative statements made by the study authors cannot be verified quantitatively	NPR
	50/50 F344 rat, diet, 105 weeks	Males: 0, 237, 474 Females: 0, 275, 550	Decreased body weight; increased focal alveolar histiocytosis in females	275	157 for increased focal alveolar histiocytosis in female rats	550	NCI (1979c)	
	50/50 B6C3F_1 mouse, diet, 107 weeks	Males: 0, 515, 1029 Females: 0, 518, 1037	Decreased body weight; increased incidence of hepatocytomegaly and nonneoplastic lesions of the liver (peliosis, hepatocellular degeneration/necrosis, and cytoplasmic vacuolation in males)	N/D	36 for increased incidence of liver peliosis in male mice	515	NCI (1979d)	
	50/50 B6C3F_1 mouse, diet, 104 weeks	Males: 0, 1640, 3480 Females: 0, 1750, 4130	Decreased body weights; increased number of foci of cellular alterations in hepatocytes in males; increased relative liver weights in mice without tumors	N/D	Not run	N/D	Inai et al. (1988); questionable dose administration	

Table 2. Summary of Potentially Relevant Data for Butylated Hydroxytoluene (CASRN 128-37-0)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Chronic	26−32/0, Syrian golden hamster, diet, 16 weeks	0, 183	Mild hyperplasia in the forestomach slightly increased; no severe or papillomatous lesions; possible interaction between chemical and feed	N/D	Not run	N/D	Hirose et al. (1986)	
	2, sex not reported, mongrel dog, diet, 1 year	0, 170, 280, 470, 500, 640, 940	No effects	940	Not run	N/D	Deichmann et al. (1955e)	
Developmental and Reproductive	2/16 F0 Wistar rat, diet, 14 weeks F1 Wistar rat (number not reported), exposed through lactation, 3 and 7 weeks	0, 500, 750, 1000 (F0)	F0 dams at ≥500 mg/kg-day: increased liver weight; ≥750 mg/kg-day: abnormal hepatocytes (enlarged, vacuolized, proliferation of ER), decreased body weight F1 pups of dams ≥500 mg/kg-day: reduced body weight	N/D	664 for decreased body weight in F0 dams	Maternal and Fetal: 500	McFarlane et al. (1997a)	
	7/50 F0 Wistar rat, diet, 14 weeks F1 Wistar rat (number and sex not reported), diet, postnatal exposure, necropsied at 22 weeks	0, 25, 100, 500 (F0) 0, 25, 100, 250 (F1)	F0 dams ≥500 mg/kg-day: increased liver weight; abnormal hepatocytes (enlarged, vacuolized, proliferation of ER) F1 pups ≥100 mg/kg-day: reduced body weight; increased liver weight; abnormal hepatocytes	Maternal: 100 F1: 25	Not run	Maternal: 500 F1: 100	McFarlane et al. (1997b)	
	60/0 Wistar rat, diet, 22 months, interim sacrifices at 1,6, 11, and 16 months; F1 rats were generated from the McFarlane et al. (1997b) study	0, 25, 100, 250	Decreased body weight; increased relative liver weight; enlarged and eosinophilic centrilobular hepatocytes; altered hepatic nodules; thyroid hyperactivity	25	Not run	100	Price (1994) as cited in OECD SIDS (2002); this study is a continuation of the McFarlane et al. (1997b) study	

Table 2. Summary of Potentially Relevant Data for Butylated Hydroxytoluene (CASRN 128-37-0)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Developmental and Reproductive	10/10 Crj:Cd-1 mouse, diet, dosed from 5−9 weeks before mating through F2 weaning F1 and F2 generations were observed for 21 days for toxicological effects	0, 29, 88, 263, 790	F1 pups: No effects F2 pups: No effects	790	Not run	N/D	Tanaka et al. (1993)	
	0/26−30 F0 JCL-ICR mouse, gavage, GD 7−13 0/19−20 F0 JCL-ICR mouse, gavage, GD 9	0, 70, 240, 800 0, 1200, 1800	F0 dams: increased spleen and kidney weight at 800 mg/kg-day F0 dam: increased mortality; increased spleen and lung weights in dams	N/D	Not run	N/D	Tokyo Metropolitan Research Laboratory of Public Health (1992b); data tables are illegible, and, therefore, the qualitative statements made by the study authors cannot be verified quantitatively	NPR
	60/60 F0 Wistar rat, diet, 13 weeks **100/100 F1 Wistar rat, 7 days/week, diet, 141−144 weeks**	**0, 25, 100, 500 (F0)** **0, 25, 100, 250 (F1)**	**Decreased maternal body weight** **F1 animals: decreased body weights**	**Maternal: 100 F1: Subchronic: 100 Chronic: 25**	**Not run**	**Maternal: 500 F1: Subchronic: 250 Chronic: 100**	**Olsen et al. (1986)**	**PS**

Table 2. Summary of Potentially Relevant Data for Butylated Hydroxytoluene (CASRN 128-37-0)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Carcinogenicity	21/0 Fisher 344 rat, diet, 76 weeks	0, 2, 6, 21, 64, 129	No tumors observed	Not Identified	Not run	Not identified	Williams et al. (1990)	
	27/0 Fisher 344 rat, diet, 110 weeks	0, 257	No tumors observed; increased mortality in both the control and treated groups after 84 weeks	Not Identified	Not run	Not identified	Williams et al. (1990)	
	57/57 Wistar rat, diet, 104 weeks	Males: 0, 52, 210 Females: 0, 54, 215	Increased pituitary gland adenomas at the low-dose in females only	Not Identified	Not run	Not identified	Hirose et al. (1981)	
	50/50 F344 rat, diet, 105 weeks	Males: 0, 64, 129 Females: 0, 66, 132	No tumors observed	Not Identified	Not run	Not identified	NCI (1979c)	
	50/50 B6C3F_1 mouse, diet, 107 weeks	0, 78, 156	Increased lung alveolar/bronchiolar carcinomas or lung adenomas at the low-dose level in females only	Not Identified	Not run	Not identified	NCI (1979d)	
	50/50 B6C3F_1 mouse, diet, 104 weeks	Males: 0, 249, 529 Females: 0, 262, 619	Increased incidence of hepatocellular adenomas at the high-dose level in males only	Not identified	Not run	Not identified	Inai et al. (1988); questionable dose administration	
	~60/0 Wistar rat, diet, 22 months, interim sacrifices at 1,6, 11, or 16 months; F1 rats generated from the McFarlane et al. (1997b) study	0, 7.1, 29, 71	No tumors observed	Not identified	Not run	Not identified	Price (1994) as cited in OECD SIDS (2002); this study is a continuation of the McFarlane et al. (1997b) study	

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Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Carcinogenicity	**60/60 F0 Wistar rat, diet, 13 weeks** **100/100 F1 Wistar rat, diet, 141−144 weeks**	**F1 Males: 0, 7.1, 28, 69** **F1 Females: 0, 6.4, 25, 62**	**Dose-related increase in hepatocellular tumors (hepatocellular adenomas and carcinomas; primarily in animals exposed for 141 weeks or more)**	**Not Identified**	**28 for increased total hepatocellular tumors (adenomas and carcinomas) in F1 male rats**	**Not identified**	**Olsen et al. (1986)**	**PS**
2. Inhalation (mg/m^3)[a]								
Subchronic	None							
Chronic	None							
Developmental and Reproductive	None							
Carcinogenic	None							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects and to a human equivalent dose (HED in mg/kg-d) for oral carcinogenic effects. All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values from animal developmental studies are not adjusted to a continuous exposure. HED = (avg. mg test article ÷ avg. kg body weight ÷ number daily dosed)$^{1/4}$.
[b]Not reported by the study author, but determined from data.
[c]PS = principal study, indicated by bold text; NPR = not peer-reviewed.

N/D = Not Determinable.

HUMAN STUDIES

Oral Exposures

The effects of oral exposure of humans to BHT have been evaluated in two case studies of intentional oral self-administration of BHT (i.e., Grogan, 1986; Shlian and Goldstone, 1986), one long-term epidemiologic study (i.e., Botterweck et al., 2000), as well as case studies following dermal exposure (i.e., Dissanayake and Powell, 1989; Flyvholm and Menne, 1990). No oral subchronic, reproductive, or developmental studies in humans were identified.

Short-term-duration Studies

Grogan (1986) described a case in which a 24-year-old female patient ingested 80 g of BHT (suspended in safflower oil) on an empty stomach. The patient voluntarily consumed the formula for BHT under the notion that it was a treatment for herpes. The patient experienced a light-headed feeling 30−60 minutes after ingestion, followed by a headache and visual and auditory hallucinations continuing for several hours. Within 1 day, she experienced slurred speech, loss of balance, and complained that sounds seemed "far away." Examination revealed dysarthria, wide-based gait, a positive Romberg test, slowed mentation without thought disorder, and dysmetria of the left (nondominant) arm. Repeat examination 8 hours later showed no abnormalities. A 6-month follow-up showed no long-term toxicity.

Shlian and Goldstone (1986) identified a trend of university students taking large doses of BHT as a treatment for genital herpes simplex virus infections. The study authors described one case in which a 22-year-old white female ingested 4 g of BHT on an empty stomach. Hours after ingestion, the patient experienced severe epigastric cramping, weakness, nausea, and vomiting, followed by dizziness, confusion, and a brief loss of consciousness. The woman was taken to the emergency room and diagnosed with gastroenteritis before being released. The next day, she was admitted to the hospital again complaining of vomiting, dizziness, epigastric burning pain, and another brief loss of consciousness. The patient's blood pressure was 110/70, with moderate orthostatic changes. She was afebrile, with a white-cell count of 7400, and was within normal limits of liver-function tests, electrolyte measurements, electrocardiography, and electroencephalography. The symptoms disappeared within a few days after she was given hydration, prochlorperazine, and antacids.

Subchronic-duration Studies

No published studies investigating the effects of subchronic oral exposure to BHT in humans have been identified for this review.

Chronic-duration Studies

Botterweck et al. (2000) performed a prospective case-cohort study of the association between BHT and stomach cancer. Analyses were based on 192 stomach cancer cases and 2035 subcohort members (consisting of both men and women) after exclusion of prevalent cancer cases at baseline and cases diagnosed in the first or second years of follow-up. The study authors used cancer and pathology registries to determine incidence of cancer during 6.3 years of follow-up time. Multivariate rate ratios of stomach cancer were computed for all variables, and trends were analyzed by likelihood ratio tests. Mean intake of BHT was 351 µg/day among subcohort members. No statistically significant association between BHT intake and stomach cancer risk was found, and the study authors noted a nonsignificant decrease in risk with increased intake.

Developmental and Reproductive Studies

Published studies investigating the developmental or reproductive toxicity of BHT via oral exposure were not identified for this review.

Other Studies

No other oral studies of BHT exposure in humans are identified for this review.

Inhalation Exposures

No inhalation studies of BHT exposure in humans are identified for this review.

Other Exposures

Dissanayake and Powell (1989) published two peer-reviewed case studies describing contact dermatitis in two leg ulcer patients treated with BHT-containing bandages. The cases included a 77-year-old man with an 18-month history of stasis ulcers being treated using paste bandages, and a 70-year-old male patient with bilateral venous stasis and intolerance to support bandages. Patch testing revealed a positive reaction (i.e., eczema) to BHT. Both patients improved when treatment with the BHT-containing bandages ceased.

Flyvholm and Menne (1990) conducted patch tests with BHT on 1336 consecutive eczema patients (consisting of both men and women). Patients were tested from September 1987 to December 1989 and were all new referrals. Patch tests were left on the skin for a 2-day period, and readings were performed after 2 days, 3 days, and 1 week. All of the patch tests with BHT were negative, at all time points. Based on these results, the study authors concluded that BHT does not cause allergic dermatitis.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to BHT have been evaluated in 3 subacute-duration- (i.e., Powell et al., 1986; Deichmann et al., 1955a,b), 4 subchronic-duration- (Fulton et al., 1980; Deichmann et al., 1955c; NCI, 1979a,b), 9 chronic-duration- (Williams et al., 1990; Hirose et al., 1993, 1981, 1986; Deichmann et al., 1955d,e; Tokyo Metropolitan Research Laboratory of Public Health, 1992a; NCI, 1979c,d; Olsen et al., 1986; Inai et al., 1988), 4 developmental and reproductive toxicity (McFarlane et al., 1997a,b; Tanaka et al., 1993; Tokyo Metropolitan Research Laboratory of Public Health, 1992b), and 5 carcinogenicity studies (Hirose et al., 1993; NCI, 1979c,d; Olsen et al., 1986; Inai et al., 1988). Many additional studies on the effects of oral exposure of animals to BHT were identified, but, because they do not instruct the POD, are excluded from this review. A list of these references is available in an accompanying separate supplemental document.

Short-term Studies

In a published, peer-reviewed, subacute study, Powell et al. (1986) investigated the oral toxicity of BHT (99.9% purity, in arachis oil vehicle) in male Wistar rats (200 g). It is unknown if the study was conducted in compliance with Good Laboratory Practice (GLP). The study authors administered 0, 25, 250, or 500 mg/kg-day BHT dissolved in arachis oil to groups of five rats per dose via gavage once per day for 7 days. Rats in the 500-mg/kg group were initially administered 750 mg/kg-day BHT for Days 1−3 and 500 mg/kg-day thereafter until study termination for an average daily dose of 607 mg/kg-day [([750 mg/kg-day × 3 days] + [500 mg/kg-day × 4 days]) ÷ 7 days total]. In a second experiment, the study authors

administered the same doses once per day for 28 days via gavage to groups of 10 rats (resulting in an adjusted high dose of 527 mg/kg-day). The study authors measured animal body weights periodically (frequency unspecified) throughout study duration. At study termination, the study authors performed histological examinations on stained sections of the rats' livers and prepared microsomal fractions for enzyme assay by homogenizing the liver tissue of each animal. Additionally, in the second experiment, liver sections were stained for two enzymes of cytochrome P-450. High-performance liquid chromatography (HPLC) was used to analyze BHT concentrations in the liver and epididymal adipose tissue. Statistical analysis of all of the endpoints measured was performed using the Student's *t*-test.

In the 7-day experiment, rats administered 250 mg/kg-day BHT had final body weights slightly lower than those of the control group (Powell et al., 1986). Body weights decreased 7 g per day on Days 1−3 when the high-dose group was fed 750 mg/kg-day BHT, but the weight was gained back beginning on Day 4 when the daily dose was reduced to 500 mg/kg-day. Lower body weights were observed in rats in the 607 mg/kg-day BHT dose group compared with the concurrent control in the 28-day study. The study authors did not observe a dose-dependent change in body weight in either experiment. A dose-related increase in relative liver weights was reported at all doses with the greatest increase (166% above controls) seen in the 607-mg/kg-day BHT group in the 7-day experiment. BHT concentrations in epididymal adipose tissue exhibited a dose-related trend in both the 28-day and the 7-day experiment (see Table B.1). Hepatic glucose-6-phosphatase activity and microsomal protein yield were statistically significantly decreased in rats in the 607 mg/kg-day BHT dose group in the 7-day experiment (see Table B.2). In the 28-day experiment, rats in the 250 and 527 mg/kg-day BHT dose groups displayed statistically significantly decreased glucose-6-phosphatase activity and increased microsomal protein yield. In addition, increased concentrations of hepatic cytochrome b_5 were observed at the 250 mg/kg-day dose level in the 7-day experiment and at the 25- and 250 mg/kg-day dose levels (see Table B.2) in the 28-day experiment. Cytochrome P-450 concentrations were 288 and 218 nmol/whole liver (wet weight) in the 7-day experiment and 475 and 462 nmol/whole liver (wet weight) in the 28-day experiment, in the 250 and 500 mg/kg-day dose groups, respectively. The study authors reported a dose-dependent increase in activities of ethoxycoumarin *O*-deethylase and microsomal epoxide hydrolase, but not in ethoxyresorufin *O*-deethylase. In both experiments, progressive periportal hepatocyte necrosis was observed only at the 500 mg/kg-day dose level (607 mg/kg-day for 7-day study and 527 mg/kg-day for 28-day study), with 2/5 and 6/10 rats displaying lesions in the 7- and 28-day experiments, respectively (see Table B.3). Morphological abnormalities in the periportal region were observed at the 250 mg/kg-day (glycogen accumulation only) and 500 mg/kg-day (607 mg/kg-day for 7-day study and 527 mg/kg-day for 28-day study) dose levels (fibrosis, hepatocyte hypertrophy, hepatocyte hyperplasia, etc.) of both experiments. The study authors further stated that hepatomegaly, as indicated by increased relative liver weight, was found at autopsy in animals receiving BHT for 7 or 28 days. However, only qualitative statements and a bar graph without quantitative data were presented for increased relative liver weight, and, therefore, this effect cannot be used to derive a reference value.

Powell et al. (1986) concluded that animals dosed with BHT at 250 mg/kg-day or greater for 28 days suffered progressive liver cell damage, as first indicated by decreased hepatic glucose 6-phosphatase activity, considered as evidence of endoplasmic reticulum disruption. Furthermore, the study authors suggested that their results may explain the increased hepatocellular carcinomas following 250 mg/kg-day administration of BHT for 2.5 years

described by Olsen et al. (1986), which the study authors stated may be caused by chronic liver damage, evidenced by findings of hepatocellular necrosis. However, decreased hepatic glucose 6-phosphatase activity is not considered a critical effect. Therefore, based on increased incidences of nonneoplastic lesions (i.e., necrosis, fibrosis, hepatocyte hypertrophy, and hepatocyte hyperplasia) of the liver, a NOAEL of 250 mg/kg-day with a LOAEL of 607 mg/kg-day is identified from the 7-day study and a LOAEL of 527 mg/kg-day from the 28-day study.

Deichmann et al. (1955) conducted a series of exposure studies using varying exposure duration, administration, and test species. The separate experiments in this peer-reviewed publication are designated with postscripts a−e. While applicable toxicity information is presented, many aspects of the methods are unreported, including animal husbandry details, examination parameters, and statistical methods. There is no statement of certification; however, this study was conducted prior to adoption of GLP. The results presented in this review are for supporting purposes because the lack of methodological details precludes use of this study in derivation of a provisional reference value.

Deichmann et al. (1955a) conducted a paired feeding experiment in male albino Wistar rats for 25 days. The specific methods and endpoints measured were not reported beyond a synthesis of the results. Five animals were given control feed or 0.8% BHT (purity not reported), and 10 animals were given 1.0% BHT for 25 days. BHT concentrations in this experiment were increased gradually with the animals receiving 0.4% (4000 ppm) BHT on Days 1−4, 0.8% (8000 ppm) on Days 5−7 (or through Day 25 for the 0.8% group), and 1% (10,000 ppm) for the duration of the study. The study did not report appropriate body-weight data for use in the dose conversion although food consumption data were reported. However, because food consumption and body weight are related parameters, values provided for male Wistar rats by EPA (1994b) for body weight (0.217 kg) and food consumption (0.02 kg/day) are used in the dosimetric calculation in the interest of consistency. The adjusted daily doses were time weighted (i.e., [(4000 ppm × 4 ÷ 25 days) + (8000 ppm × 3÷ 25 days) + (10,000 ppm × 18 ÷ 25 days)] × Food Consumption per Day × [1 ÷ Body Weight] × [days dose ÷ total days]). Average daily food consumption was 12.3 g (84% of control) and 12.8 g (87% of control) for the 0.8%- and 1.0%-dose groups, respectively. The corresponding adjusted daily doses are 678 and 811 mg/kg-day. Despite the decreased food intake in both dose groups compared to the control group, mean total body-weight gain over the study period increased (120% of control) in the 0.8%-dose group, while it declined greatly (43% of control) in the 1.0%-dose group. Besides body-weight gain and food consumption changes, no other BHT-related effects were reported. Based on the diminished body-weight gain as compared to controls, a $LOAEL_{ADJ}$ of 811 mg/kg-day is identified with a corresponding $NOAEL_{ADJ}$ of 678 mg/kg-day.

In one subacute experiment among a series of experiments by Deichman et al. (1955b) study, groups of four mongrel dogs (sex, age, and weight not reported) were administered doses of 0-, 1.4-, 2.1-, 3.2-, or 4.7-g/kg BHT in the diet 2 days per week for the first 2 weeks followed by 3 days per week for the remaining 2 weeks in a 28-day study period. The corresponding daily doses (adjusted for days dosed/total days) are 0, 600, 900, 1371, and 2014 mg/kg-day. All of the animals exposed to BHT showed various degrees of diarrhea within 1−3 hours of exposure. This effect was delayed over the course of the experiment, with diarrhea occurring 24 hours after exposure by Week 4 of the study (data not reported). The study authors reported no other clinical signs of toxicity. Gross pathology following sacrifice at the study termination showed

hemorrhages, edema, and congestion in the lungs, which were attributed to the method (air embolism) used for sacrifice (data not reported). No BHT-related pathologies were observed in the gastrointestinal tract. Based on the study author's description of no chemical-related effects, a $NOAEL_{ADJ}$ of 2014 mg/kg-day is identified. This study will not support derivation of a subchronic p-RfD due to a lack of methods and data reporting.

Subchronic-duration Studies

In a published, peer-reviewed study, Fulton et al. (1980) evaluated the effects of BHT exposure on selected tissues in Wistar rats. Neat BHT (purity not specified) was administered to 10 male Wistar rats (weighing 36−63 g) at doses of 0, 0.02, 0.1, 0.5, or 0.75% of diet for 8 weeks. The study did not report appropriate body-weight data for use in the dose conversion although food consumption data were reported. However, because food consumption and body weight are related parameters, values provided for male Wistar weanling rats by EPA (1994b) for body weight (0.053 kg) and food consumption (0.008 kg/day) are used in the dosimetric calculation in the interest of consistency. The corresponding daily doses are 0, 30, 151, 755, or 1132 mg/kg-day. Food and water were available ad libitum. Food intake and body weights were recorded daily during treatment but not reported. After 8 weeks, animals were sacrificed, and liver weights were recorded. In addition, femoral bone marrow samples were obtained from each animal. Erythrocyte counts were taken as an indicator of liver dysfunction. The proximal ileum section of the small intestine was evaluated for villus height, crypt of Lieberkühn depth, and goblet cell count. Study authors performed statistical analysis using a partial correlation for multivariant data. No information was provided regarding GLP compliance.

Table B.4 provides initial body weights, total food intake, body-weight gain, liver weight, and relative liver-weight data reported by Fulton et al. (1980). Study authors reported significantly higher food intake in the 0.02%- and 0.1%-dose groups and no statistical difference in food consumed between other dose groups and controls. Total body-weight gain in the 0.5%- and 0.75%-dose groups was significantly lower than that of controls, and body weight at sacrifice decreased in a dose-related manner. Study authors stated there was no dose-related trend in mean absolute liver weights but reported a statistically significant decrease in liver-to-body-weight ratios as the level of BHT in the diet increased. However, this conclusion is not supported by the data presented in the study report, which shows an increase with dose in the liver-to-body weight ratio, as presented in Table B.4. Despite comments stating statistical significance, statistical results associated with specific dose levels were not reported. An independent statistical analysis could not be performed because only group means, without standard deviations, were presented by the study authors.

Table B.5 presents the results of the ileal biopsy. Ileal biopsies revealed a nonsignificant shortening and broadening of the villi with increasing dose and a statistically significant (reported by the study authors) dose-related decrease in the depth of the crypts. Study authors observed lower goblet cell counts in dose groups of 0.10% and higher but did not report statistical analysis. Study authors observed no significant differences in the number of immature erythrocytes between dose groups and controls upon examination of the femoral bone marrow. The $LOAEL_{ADJ}$ for Fulton et al. (1980) is identified to be 30 mg/kg-day in male rats based on ≥10% increase in relative liver weight, considered to be biologically significant. Because the LOAEL identified is the lowest dose administered, a NOAEL cannot be identified.

In a 90-day albino Wistar rat study (Deichmann et al., 1955c), two different grades of BHT (99.7 and 98.8%) were added to the diet for exposure concentrations of 0.2, 0.5, 0.8, and 1.0% BHT in 1.0% lard, resulting in eight exposure groups of three animals per group. Control groups included feed alone as well as feed supplemented with 1.0% lard. Two additional groups of rats were exposed to increasing concentrations of BHT (0.2% initially) at intervals of 3−4 days until a BHT concentration of 1.5% was reached. The sex, age, and weights of the rats used were not reported. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for Wistar rats by EPA (1994b) for body weight (0.1865 kg for both sexes) and food consumption (0.018 kg/day for both sexes) are used in the dosimetric calculation. It is also assumed that because the details of the ramping method of dosing are not provided, the doses were consistent over the exposure duration. The corresponding adjusted daily doses are 193, 483, 772, 965, and 1448 mg/kg-day, respectively. The specific endpoints examined are not specified, along with many methodological details such as the age and weight of animals at study initiation. No differences in animals treated with the two BHT grades were observed. No effects were reported in the animals exposed to 193 mg/kg-day. Increased mortality was observed at the 483 mg/kg-day and greater doses (1/6, 2/6, 1/6, at the 483, 772, and 965 mg/kg-day doses, respectively, at 12 days, 4 and 5 weeks, and 13 weeks, respectively). Animals in the 1448-mg/kg-day group refused to eat, and mortality in this group was 4/6 animals with one animal found dead at 4, 4, 9, and 11 weeks, respectively. For this study, a NOAEL of 193 mg/kg-day is identified, but no LOAEL can be determined because the next highest dose of 483 mg/kg-day is an FEL.

NCI (1979a) conducted a rat subchronic-duration study, which is summarized here, as well as a mouse subchronic-duration study, which is summarized separately. Researchers administered neat BHT (purity 99.9%) by diet to 5 F334 rats per sex per dose at levels of 0, 6200, 12,500, 25,000, or 50,000 ppm for 7 weeks and then observed animals for 1 week. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for F344 rats by EPA (1994b) for body weight (0.18 kg for males and 0.124 kg for females) and food consumption (0.018 kg/day for males and 0.014 kg/day for females) are used in the dosimetric calculations. The corresponding adjusted daily doses are 0, 620, 1250, 2500, and 5000 mg/kg-day for males and 0, 700, 1411, 2822, and 5645 mg/kg-day for females. Feed and water were supplied continuously ad libitum for the duration of the study. The study authors weighed each animal prior to the study period and then twice weekly until study termination; however, body-weight data were not reported in the study. All of the animals were sacrificed using CO_2 and necropsied at the conclusion of the study. The study authors conducted histopathological examinations (specific endpoints unreported) of each animal following necropsy. The study authors used a 10% depression in body weight as a major criterion for the estimation of maximum tolerated doses (MTDs). Least squares regression of mean body weight per number of days was applied to estimate the final mean body weights of each group. Study authors plotted probits of the percentage weights of each dose group at Day 49 relative to those of the corresponding control groups against the logarithms of the doses. Study authors then used fitted least squares regression to estimate the doses that induced 10% reduction in body weight.

NCI (1979a) observed decreased survival rates with increasing dose and 100% mortality rate for both males and females at the highest dose level (see Table B.6). Decreased mean body weights also were observed with increasing dose, and this trend was more pronounced in males than females. The 10% depression threshold in mean body weight was recorded at

620 mg/kg-day in males and 1411 mg/kg-day in females. Study authors also reported slight increased hematopoiesis in rats treated with 1250 mg/kg-day in males and 1411 mg/kg-day in females (date not shown). Based on a 10% weight loss in male rats, a $LOAEL_{ADJ}$ of 620 mg/kg-day is identified. This LOAEL corresponds to the dose at which males lost at least 10% mean body weight. Because the dose at which the LOAEL is reported was the lowest dose administered, a NOAEL cannot be identified.

Parallel to the subchronic-duration study in rats, NCI (1979b) published results from a subchronic-duration study in which they administered groups of five $B6C3F_1$ mice per sex per dose dietary concentrations of 0, 3100, 6200, 12,500, 25,000, or 50,000 ppm BHT for 7 weeks, followed by 1 week of observation. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for $B6C3F_1$ mice by EPA (1994b) for body weight (0.0316 kg for males and 0.0246 kg for females) and food consumption (0.0057 kg/day for males and 0.0048 kg/day for females) are used in the dosimetric calculation. The corresponding adjusted daily doses are 0, 559, 1118, 2255, 4510, and 9019 mg/kg-day for males and 0, 605, 1210, 2439, 4878, and 9756 mg/kg-day for females. The study was performed using the same methods of exposure, examination parameters, and statistical analysis as the subchronic-duration study in rats described previously (NCI, 1979a).

NCI (1979b) reported mortality in the males (1/5) and females (4/5) in the high-dose group (see Table B.7). Study authors observed a decreasing trend in mean body weight with increasing dose in males. A similar trend was also observed in females, except in the highest dose group, in which the remaining single surviving female had a mean body weight of 97% of control. Males and females in the lowest dose group experienced a threshold decrease in mean body weight. In addition to body-weight changes, the study authors also reported observing findings of centrilobular cytoplasmic vacuolation in the livers of male animals in the highest dose group (quantitative data not reported). Based on a 10% loss in body weight in male mice, a $LOAEL_{ADJ}$ of 559 mg/kg-day is identified. Because this was the lowest dose administered, a NOAEL cannot be identified.

Chronic-duration Studies

In a peer-reviewed publication, Williams et al. (1990) investigated the oral toxicity of BHT (99% purity determined by thin layer chromatography [TLC]) in male F344 rats in two studies. The study authors administered a basal diet (NIH-07) containing 100, 300, 1000, 3000, or 6000 ppm BHT to groups of 21 six-week-old male (100 g) rats for 76 weeks in one study, and a single 12,000 ppm BHT dose to groups of 27 eleven-week-old male (200 g) rats for 110 weeks in the second study. A separate group of 36 six-week-old (100 g) and 27 eleven-week-old male (200 g) rats served as control groups, respectively. Appropriate body-weight data and food consumption data for dose conversion were not provided in the studies. Therefore, average values provided for male Fisher 344 rats by EPA (1994b) for body weight (0.38 kg) and food consumption (0.03 kg/day) are used in the dosimetric calculation. The adjusted daily doses are 8, 24, 79, 237, and 474 mg/kg-day for the 76-week study and 0 and 947 mg/kg-day for the 110-week study. Four rats from each group treated for 76 weeks were sacrificed 12, 36, 48, and 76 weeks after study commencement for hepatocellular foci analysis. The study authors recorded body weights every 4 weeks. Complete autopsies on all animals were performed at study termination (i.e., 76 and 110 weeks). The study authors recorded liver weights, took slices from each lobe for staining, and tested for iron to identify iron-deficient lesions. Histopathological examinations were performed on the liver and other unspecified organs.

Morphological analysis of altered cellular foci in the liver was performed by microscopic quantization of the number of foci per cm^2, adjusted, and presented to be relative to the total area of the liver section. Statistical analysis was performed using the Student's *t*-test to analyze differences between groups and Fisher's exact probability test to analyze neoplasm incidence. No information is presented regarding GLP compliance.

Williams et al. (1990) reported no mortalities in rats administered BHT for 76 weeks. Rats in the control and treatment groups exposed for 110 weeks displayed increased mortality beginning at Week 84, with 11/27 and 9/27 mortalities, respectively, noted at study termination. Body-weight gain was reduced significantly in rats administered 3000- (89% of control) and 6000-ppm BHT (89% of control) for 76 weeks and in rats administered 12,000-ppm BHT (89% of control) for 110 weeks, relative to controls (see Table B.8). Increased absolute liver weights were observed in rats in the 76-week 6000-ppm dose group (32% over control), relative to controls. Relative liver weight (per 100 g body weight) was biologically significantly increased (11% over control) in the 3000-ppm dose group and statistically and biologically significant (50% over control) in the 6000-ppm dose group. The size of hepatocellular foci increased slightly—but not significantly—in the 6000-ppm dose group (76 weeks) and decreased slightly—but not significantly—in rats in the 12,000-ppm group (110 weeks) (see Table B.9). The study authors observed mild hyperplasia in the stomachs of all examined groups, including control (controls, 6000 ppm, controls, and 12,000 ppm) (see Table B.10). Incidence was not dose related, and no other gastric lesions were identified in any of the groups.

Neoplasm incidence was measured by Williams et al. (1990) and was not significant in either study and the study authors concluded that BHT alone is not a carcinogen. For the 76-week study, the biologically significant (≥10%) increase in relative liver weight in animals exposed to 3000- and 6000-ppm BHT supports a $LOAEL_{ADJ}$ of 237 mg/kg-day and a corresponding $NOAEL_{ADJ}$ of 79 mg/kg-day. For the 110-week study, a $LOAEL_{ADJ}$ of 947 mg/kg-day is identified based on decreased body weight (≥10%). Because this is the only dose tested, identification of a NOAEL is precluded.

Hirose et al. (1993) conducted a published, peer-reviewed 36- to 40-week study in which they exposed groups of F344 male rats (5 weeks of age, weight unreported), obtained from Charles River Japan, Inc. (Kanagawa, Japan), to a series of carcinogens, followed by treatment with antioxidants including BHT. The study authors also exposed rats to BHT (>98% purity) or other potential antioxidants individually. Hirose et al. (1993) exposed a group of 12 animals to 0.7%- (7000-ppm) BHT in powdered basal diet for 36 weeks, whereas 11 control animals received only basal diet. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for F344 male rats by EPA (1994b) for body weight (0.18 kg) and food consumption (0.018 kg/day) are used in the dosimetric calculation. The adjusted daily doses are 0 and 700 mg/kg-day. Rats were housed five to a cage in plastic cages at 24 ± 2°C on 12-hour-light/dark cycle. Their basal diet consisted of Oriental MF (Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum. The animals were weighed every 2−4 weeks during treatment with BHT. Surviving animals with tumors in any of the experimental groups were included for analysis. Animals were sacrificed and autopsied after 36 weeks of treatment. At study termination, liver and kidneys were weighed, and portions were removed for immunohistochemical staining for glutathione *S*-transferase placental form (GST-P). The number of lung lesions and the number and area of GST-P-positive foci were measured. After the data collection, Student's *t*-tests and Fisher's exact probability tests were

used in the statistical analysis of the data. No information on GLP compliance was provided by the study authors.

Hirose et al. (1993) reported statistically significantly decreased body weights (87% of control) in rats treated with BHT (n = 12) compared to those of the control groups (n = 11) (see Table B.11). Relative liver weight was significantly greater in rats treated with BHT (176%). The relative kidney weight also was increased significantly (128%) (see Table B.11). However, 3 of 12 rats treated only with BHT died of abdominal hemorrhage. No other treatment-related effects of BHT were reported by the study authors, and no further data were provided.

As noted above, Hirose et al. (1993) observed that in the only dose of BHT administered (700 mg/kg-day or 0.7% in powdered food), 3 of the 12 rats died of hemorrhage. Due to this frank effect, an FEL of 700 mg/kg-day is identified. Because this is the only dose tested, identification of a NOAEL is precluded.

Hirose et al. (1981) published a 2-year, peer-reviewed, chronic-duration study investigating the toxicity of BHT. The study authors administered a basal diet containing 0%, 0.25% (2500 ppm), and 1% (10,000 ppm) BHT (purity not reported) in pellet form to 36, 57, and 57 seven-week-old male and female Wistar rats (100−200 g at study initiation), respectively. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for Wistar rats by EPA (1994b) for body weight (0.462 kg for males and 0.297 kg for females) and food consumption (0.034 kg/day for males and 0.025 kg/day for females) have been used in the dosimetric calculation. Adjusted daily doses are 0, 184, and 736 mg/kg-day for males and 0, 210, and 842 mg/kg-day for females. The study authors recorded body weights weekly for the duration of the study and measured food consumption at unspecified regular intervals. After the treatment period, study authors sacrificed animals and collected blood samples for measurement of clinical chemistry parameters including red and white blood cell counts, hemoglobin, hematocrit, glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatatase, cholinesterase, γ-glutamyl transpeptidase (γ-GTP), total protein, albumin/globulin, thymol turbidity, total cholesterol, triglyceride, β-lipoprotein, blood urea nitrogen, creatinine, uric acid, total bilirubin, sodium, potassium, chloride, and inorganic phosphate. They also measured organ weights for the liver, spleen, and kidneys and performed histological examinations of the liver, pancreas, mammary gland, uterus, pituitary gland, adrenal gland, lung, thyroid, and kidneys. Study authors did not provide any information on GLP compliance. Statistical analysis did not include rats surviving less than 69 weeks. Study authors performed statistical analysis using the chi-square test when comparing groups and the Student's t-test to analyze variance and differences between means.

Hirose et al. (1981) reported increased mortality in high-dose males from Week 96 until the end of the study (approximately 60% when compared to 35% of controls, estimated using graph-digitizing software). Mortality prior to Week 96 was 28% (10/36), 25% (14/57), and 33% (19/57) in males and 11% (4/36), 19% (11/57), and 10.5% (6/57) in females in the 0-, 0.25-, and 1%-dose groups, respectively. Dietary administration of BHT significantly decreased body weight in male rats with recovery occurring by Week 36 in the low-dose group and by Week 60 in the high-dose group. Females administered 0.25%-BHT experienced a significant decrease in body-weight gain at Weeks 12 and 48, and the females administered 1%-BHT also experienced a decrease throughout the study. Most animals recovered over the course of the study period, as reported by the study authors, although the body-weight data, presented graphically by the study

authors, cannot be confirmed by graph-digitizing software used for this review. Males and females in all BHT dose groups displayed increased absolute (data not shown) and relative liver weights at study termination (see Table B.12). Average relative liver weights were 2.5, 4.1 (64% over control), and 3.7 g/100 g (48% over control) body weight in males and 2.8, 2.9 (4% over control), and 3.5 g/100 g (25% over control) body weight in females in the 0-, 0.25-, and 1%-dose groups, respectively. Male rats did not display decreased spleen weights, but average relative spleen weights were decreased (62 and 68% of controls) per 100 g body weight in females in the 0.25- and 1%-dose groups compared to controls. Individual animal spleen weights and the variance of the mean or absolute spleen-weight data were not reported. No other organ-weight changes were reported. Treatment-related effects seen during hematological and serum biochemistry evaluations are provided in Table B.13. At the end of the study, females in both treated groups had increased red blood cell (RBC) counts. White blood cell (WBC) counts were decreased at the low dose in males and in females at the low- and high-dose levels (data not reported by study authors). Changes in RBC and WBC counts were not dose dependent. In the 0.25- and 1%- groups, serum triglyceride in males was significantly lower (140 and 137 mg/dl, respectively, compared to 180 mg/dl in the control group), γ-GTP in males was significantly higher (3.8 and 4.4 mU/dl, respectively, compared to 2.8 mU/dl in the control group), and total cholesterol in females was significantly higher (99.2 and 112 mg/dl, respectively, compared to 73.4 mg/dl in the control group). Total cholesterol in males was significantly higher only in the 0.25%-group (94.8 mg/dl compared to 83.1 mg/dl in the control group). No other changes in hematology or serum biochemistry were reported. While a significant increase in pituitary adenomas (13%) was reported in the low-dose females, the effect was reportedly not related to chemical exposure, as the incidence was not as high in the high-dose females (11.8%). No treatment-related nonneoplastic or neoplastic lesions were observed in BHT-treated animals although nonsignificant increases were reported in the incidence of total tumors, which were higher in the 0.25%-dose group than the 1%-dose group, and, therefore, reported not to be dose related. In males, there was a small increase in tumors of the pancreas of treated animals (carcinomas: 2.6% in the 1.0%-dose group as compared with 0% in the 0.25%- and control groups; islet-cell adenoma: 2.3% in the 0.25%-dose group, and 5.3% in the 1.0%-dose group, as compared to 0% in the control group). Female animals showed small increases in the incidence in tumors of the liver (6.5% in the 0.25%-dose group and 5.9% in the 1%-dose group, as compared to 0% in the control group) (see Table B.14).

Statistically significant changes were observed in levels of triglycerides in male rats, γ-GTP in male rats, and total cholesterol in both sexes. These enzyme effects are supported by increased relative liver weights, indicating liver injury. Changes in the RBC and WBC levels, while significant, are not supported by examination of the hematopoietic system for morphological abnormalities by the study authors. Total tumor incidence was reported by the study authors as not related to BHT treatment because dose-related increases in tumors were not observed. Due to the increased mortality representing a frank effect in the high-dose treated males (60%) as compared to controls (35%), the endpoints measured at this level cannot be considered. A $LOAEL_{ADJ}$ of 184 mg/kg-day is identified based on a 64% increase in relative liver weight in male rats, considered to be biologically significant; a NOAEL cannot be identified because liver effects were seen at the lowest administered dose.

Two chronic-duration studies, peer-reviewed and published by Deichmann et al. (1955d,e), were performed according to the same methods as previously described for the subacute and subchronic studies. These studies were performed prior to adoption of GLP. In a

2-year chronic toxicity study (Deichmann et al., 1955d), study authors administered groups of 15 male and 15 female albino Wistar rats 0.2% (2000 ppm), 0.5% (5000 ppm), 0.8% (8000 ppm), and 1.0% (10,000 ppm) BHT (purity not reported) in 1% lard in diet. The study authors included a control group of rats fed 1% lard and a group exposed to 0.5% (5000 ppm) BHT dissolved in lard and heated at 150º C for 30 minutes. This experiment was conducted to assess the impact of heating on the stability and toxicity of BHT. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for Wistar rats by EPA (1994b) for body weight (0.462 kg for males and 0.297 kg for females) and food consumption (0.034 kg/day for males and 0.025 kg/day for females) are used in the dosimetric calculation. The adjusted daily doses are 147, 368, 589, and 736 mg/kg-day for males and 168, 421, 673, and 842 mg/kg-day for females. Body weights were measured once per week until stable (time point unspecified) at which point body weights were determined once per month. Measurements of blood counts and hemoglobin were taken "at intervals" in randomly selected animals although the study authors reportedly selected animals that appeared less healthy (Deichmann et al., 1955d). Study authors conducted gross pathological examination on all animals surviving until study termination including recording weights of major organs and preparing tissue for micropathological analysis. Microscopic analysis of organs (brain, lung, heart, spleen, liver, kidneys, and enteric tract) involved paraffin embedding of tissue, followed by staining with hematoxylin, eosin, and/or Sudan III.

Deichmann et al. (1955d) reported mortalities in various groups (data were not reported by study authors), but concluded that they were not dose-related. There was no noteworthy effect on the number of erythrocytes and leucocytes or the hemoglobin concentration (see Tables B.15 and B.16). There were also biologically significant increases in relative liver and kidney weights at some doses in both male and female rats. Specifically, there was an 11% increase in relative kidney weight in female rats at a dose of 168 mg/kg-day, see Table B.16. Pathological examinations indicated no dose-related effects. Both males and females suffered from pneumonia, but incidence was higher in the low-dose groups and controls compared to the high-dose group. Similarly, neoplasms were identified, however incidence in the control group (4/6 females, 2/6 males), and a lack of incidence in the high-dose group, indicated that neoplasms were not related to dosing. There were no abnormalities identified during pathological examinations of animals in the 1%-dose group. A $LOAEL_{ADJ}$ of 168 mg/kg-day (0.2-dose group in females) is identified for causing a biologically significant increase in relative kidney weight. Because this was the lowest dose administered, a NOAEL cannot be identified.

In an unpublished (not peer-reviewed) chronic toxicity study to examine changes in growth, mortality, lifespan, and appearance of tumors (Tokyo Metropolitan Research Laboratory of Public Health, 1992a), male and female JCL:S-D rats were fed BHT (purity not reported) in the diet at doses of 0, 2.5, 10, or 160 mg/kg-day (40/sex/dose group) for 3, 6, 12, or 24 months or throughout the lifespan (total number of weeks not specified). BHT was added to powder feed CE-2 of Nihon Clea Co., Ltd. The animals were housed individually in cages suspended on belt-type racks that were equipped with an automatic water supply system. Diet and water were given ad libitum. Environmental conditions were described by a temperature of 25 ± 1°C, 55 ± 5% humidity, and a 13-hour light/11-hour dark cycle. No information is provided regarding GLP compliance.

The Tokyo Metropolitan Research Laboratory of Public Health (1992a) observed behavior daily; body weight and food intake were measured in the lifespan group weekly for the

first 3 months, every 2 weeks from 4−8 months of feeding, then every 4 weeks until 24 months of feeding. At the end of the 3-, 6-, and 12-month feedings, animals were sacrificed from all three groups, and liver, kidney, heart, spleen, thyroid, and caecum weights were recorded. Additionally, study authors performed hematological examination, serum biochemical examination, urinalysis, and histological examination of tissues. After necropsy of animals in the 24-month feeding group, study authors recorded weights of heart, liver, kidney, spleen, pituitary, thyroid, adrenal, testis, prostate, ovary, and uterus. Study authors also recorded mortality, and completed hematological examination, serum-biochemical examination, urinalysis, and histological examination of tissues and neoplasms. For the lifespan group, detailed methods of observation were not provided in the text of the report. However, review of the study tables indicates that the same observations and measurements were recorded as those for the 24-month feeding group. Animals in moribund condition were sacrificed and some (unspecified) examinations were performed. Rats found dead were autopsied, and histological examinations of organs, tissues, and neoplasms were conducted. Statistical analysis of results was performed using the Student's *t*-test and the rank sum test.

The Tokyo Metropolitan Research Laboratory of Public Health (1992a) reported no noticeable differences in behavior between the control group and each of the treated groups. For the lifespan feeding group, body weights and rates of body weight change were presented in a report table, but most values in that table were illegible. Food intake rates for the lifespan feeding group were presented in report tables, but values in those tables were generally illegible. However, study authors reported that there were no noticeable differences in mean daily food intake in each treated group compared to the control for animals in the lifespan feeding group.

Pages of the Tokyo Metropolitan Research Laboratory of Public Health (1992a) report containing tables and figures with mortality data for the 24-month and other durations appear to be missing. However, the study authors indicated that there were no treatment-related increases or decreases in mortality for the 3-, 6-, or 12-month feeding groups. Males and females in the 24-month feeding group experienced increased mortality compared to controls—mortality in the 160-mg/kg-day dose group was noted to be statistically significantly higher compared to the control (using the Fisher direct probability calculation). Mortality in the lifespan feeding group was not significantly different than controls.

The Tokyo Metropolitan Research Laboratory of Public Health (1992a) described findings of the urinalyses, hematological examinations, and macroscopic examinations during necropsy in the report; however, tables with results are missing from the study report. The study authors stated that there were no marked differences between the control and each BHT-treated group in these parameters. Study authors present findings of the serum-biochemical examination in the text of the study report; however, tables with results are missing from the report. Statistically significant results were reported as follows for specific tests.

- Serum K^+ was significantly elevated compared to controls in the 3-month feeding group for males at 2.5, 10, and 160 mg/kg-day and females at 160 mg/kg-day. It was suggested by the study authors that significantly increased K^+ in both sexes at 160 mg/kg-day indicates the effect of BHT at an early stage of feeding.
- For serum cholesterol, slightly higher values were generally observed in treated rats until the 12-month feeding. Cholesterol was significantly elevated in the 12-month

feeding group females at 2.5 mg/kg-day, but was significantly lowered in the 24-month females at 2.5 mg/kg-day.

- Transaminase activities (i.e., GOT and GPT) were significantly lower in the 6-month feeding group males at 160 mg/kg-day compared to controls.

The 12-month feeding group males at 160 mg/kg-day showed higher values in all biochemical examinations compared to mean values; however, study authors suggested that this was due to abnormally high values in one animal (Tokyo Metropolitan Research Laboratory of Public Health, 1992a). The study authors also observed abnormal values for some examinations in the 24-month feeding group, but none were statistically significant. Results of organ weight measurements are described in the study report; however, tables with results are missing from the report. In the 160-mg/kg-day dose group, the study authors observed a trend for increased liver weight in each feeding group related to BHT intake. Histopathological findings in all feeding groups were presented in report tables; however, values in those tables were illegible. In the text, the study authors noted histological changes were believed to be related to BHT intake. In the 160-mg/kg-day dose group, there was slight swelling of hepatic cells (after 3 months) and aggregation of basophilic substances at the hepatic cytoplasmic peripheral region (after 6 months) but these findings were not observed in the 24-month and lifespan feeding groups. There was vacuolized degeneration of renal tubule epithelium, but the dose levels at which this was seen were not specified.

The Tokyo Metropolitan Research Laboratory of Public Health (1992a) did not observe tumors in the 3-, 6-, or 12-month feeding groups. Tumors that occurred relatively frequently in the 24-month and lifespan groups included: mammary gland in females, pituitary in males and females, and adrenal gland in males; all tumors were observed in each BHT-treated group as well as the control group. Other malignant tumors occurred sporadically (one or two cases) in other tissues, including the abdominal muscle, kidney, skin and subcutaneous tissue, thoracic cavity, abdominal cavity, pancreas, intestinal wall, and uterus.

At the highest dose (160 mg/kg-day BHT), the Tokyo Metropolitan Research Laboratory of Public Health (1992a) noted a tendency toward increased liver weight, serum cholesterol, and serum K^+. Histological changes in the liver and kidney were also observed at this dose. However, there were no BHT-related changes in the quantity of food intake, body-weight gain, mortality, and mean lifespan. The study authors also believed that BHT did not cause induction of malignant tumors and indicated that their occurrence in treated rats happened spontaneously. The study authors concluded that ingestion of BHT in feed (at 2.5, 10, or 160 mg/kg-day) did not cause harmful effects on the study animals. As mentioned several times in the summary of this study, the data tables are barely legible and therefore the qualitative statements made by the study authors cannot be verified quantitatively, making this study unusable for derivation of a subchronic p-RfD.

In a published, peer-reviewed, chronic-duration oral study, NCI (1979c,d) examined toxicity and carcinogenicity of BHT on F344 rats and $B6C3F_1$ mice. The chronic rat portion of the study (NCI, 1979c) is summarized here, and the chronic mouse portion (NCI, 1979d) is summarized separately. Neat BHT (99.9% purity) was administered by diet to 50 six-week-old animals per sex per dose at doses of 0, 3000, or 6000 ppm for 105 weeks. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for F344 rats by EPA (1994b) for body weight (0.38 kg for

males and 0.229 kg for females) and food consumption (0.03 kg/day for males and 0.021 kg/day for females) are used in the dosimetric calculation. Corresponding adjusted daily doses are 0, 237, or 474 mg/kg-day for males and 0, 275, or 550 mg/kg-day for females. Study authors did not report whether or not they adhered to GLP standards. BHT was mixed with Wayne® Sterilizable Lab Meal and made available ad libitum. Study authors examined animals twice daily for sick, moribund, and tumor-bearing animals and performed clinical examinations and palpations for masses each month. Animals were weighed at least once per month. The study authors made peripheral blood smears for each animal "whenever possible." Upon termination of the study, surviving animals were sacrificed using CO_2 and necropsied. All animals found dead were necropsied as well, except in cases of advanced autolysis or cannibalism.

Study authors of the NCI (1979c) study used the Carcinogenesis Bioassay Data System to record and process data, using data elements recommended by the International Union Against Cancer. Microscopic pathological parameters included skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and non glandular), small and large intestine, kidney, urinary bladder, pituitary gland, adrenal gland, thyroid, parathyroid, testes, prostate, uterus, ovaries, brain (cerebrum and cerebellum), and all tissue masses. Study authors based all statistical tests on animals that survived at least 52 weeks or until the first tumor at a particular site was discovered. The study authors used the product-limit procedure of Kaplan and Meier to estimate probabilities of survival. They performed an analysis of dose-related effects on survival between two groups by the method of Cox and tested for trend using Tarone's extension of the Cox method. Study authors used the Fisher exact test to compare tumor incidence between dose groups and controls and applied the Bonferroni inequality correction when testing multiple groups. The study authors tested for linear trend in proportions using the Cochran-Armitage test. They used life-table methods to analyze the incidence of tumors, comparing survival curves using the method of Cox and Tarone's extension. The study authors computed 95% confidence intervals for the relative risk of each dose group compared to the control from the exact interval on the odds ratio.

Study authors noted that treatment in the NCI (1979c) study resulted in dose-related decreases of mean body weight in both males and females (no data provided). The Tarone test for dose-related mortality did not provide significant results for males or females although mortality rates at both dose levels were lower than controls for both sexes (see Table B.17). Histopathological examination revealed an apparent dose-related incidence of focal alveolar histiocytosis in the lungs of males and females (see Table B.18) although the study authors reported no statistical analysis. Independent statistical analysis performed for this review (χ^2 test) indicated a significant increase in focal alveolar histiocytosis in female rats at the high-dose level. Other lesions commonly seen in aged F344 rats were observed but were not dose-related. The Cochran-Armitage test for positive dose-related incidence of tumors and the Fisher exact test comparing incidence of tumors in dose groups to controls were not significant for all examination parameters for each sex. However, the study authors observed significant results in the negative direction for incidence of adenomas of the pituitary gland in high-dose females (see Table B.19). No positive dose-related trends were apparent in primary tumors, benign tumors, or malignant tumors (see Table B.20). Study authors noted that the confidence interval for each measured tumor incidence, except that for incidence of adenomas in the pituitary gland of high-dose females, displayed an upper limit greater than one, indicating the possibility of tumor induction by BHT that could not be detected in this test.

Given the histopathological and statistical findings, NCI (1979c) concluded that BHT did not induce neoplastic or nonneoplastic lesions and, thus, it is not carcinogenic in the F344 rat at the doses administered in this study. Based on the increased incidence of alveolar focal histiocytosis in female rats exposed to BHT, a $LOAEL_{ADJ}$ of 550 mg/kg-day is identified, with a corresponding $NOAEL_{ADJ}$ of 275 mg/kg-day. The data for increased incidence of alveolar focal histiocytosis in female rats are further evaluated with the BMDS modeling program for determination of a POD for the chronic p-RfD.

Parallel to the NCI (1979c) study involving rats, NCI (1979d) published a peer-reviewed chronic-duration study in which study authors administered BHT (99.9% purity) in the diet at concentrations of 0, 3000, or 6000 ppm to 50 six-week-old $B6C3F_1$ mice per sex per dose for 107 weeks. Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for $B6C3F_1$ mice by EPA (1994b) for body weight (0.0373 kg for males and 0.0353 kg for females) and food consumption (0.0064 kg/day for males and 0.0061 kg/day for females) are used in the dosimetric calculation. Corresponding daily doses are 0, 515, or 1029 mg/kg-day for male mice and 0, 518, or 1037 mg/kg-day for female mice. Study authors applied administration methods, examination parameters, and statistical analyses as described in the 105-week NCI (1979c) study on rats. No information was provided regarding GLP compliance.

Treatment resulted in dose-related reduction of mean body weights of males and females (data not shown) (NCI, 1979d). In females, the Tarone test for dose-related mortality rate was not significant; however, the test was significant for males in the negative direction (i.e., treated lived longer then controls). Table B.29 displays the data for survival of male and female mice. Histopathological examination revealed a high incidence of multiple proliferative lesions of the liver and lungs in both sexes. Lesions also occurred in the lacrimal gland but were only examined when grossly apparent and, thus, cannot be evaluated in relation to BHT treatment (see Table B.30). Statistical tests for dose-related increased incidence of the liver adenoma and carcinoma were only significant for hepatocellular carcinomas at the highest dose in males. Dose-related and statistically significant increased incidences of hepatocytomegaly and other nonneoplastic liver lesions including peliosis, hepatocellular degeneration/necrosis, and cytoplasmic vacuolation, were observed in males but not in females. Incidence of alveolar/bronchiolar carcinomas or adenomas in low-dose females was significantly higher than the control, but the Cochran-Artmitage test of trend was not significant because a lower incidence was seen with the high dose. Study authors found a significant dose-related trend in incidence of adenomas of the lacrimal gland in males, but results of the Fisher exact test were not significant. Cochran-Armitage and Fisher exact tests for all other examination parameters were not significant. NCI (1979d) reported that no dose-related tumors were observed following treatment with BHT (see Table B.31).

NCI (1979d) concluded that BHT was not carcinogenic in $B6C3F_1$ mice of either sex. However, there were dose-related increases in nonneoplastic lesions in livers of male mice. Based on these observations, a $LOAEL_{ADJ}$ of 515 mg/kg-day is identified. Effects were observed at the lowest dose administered, precluding identification of a NOAEL. The data for these effects (i.e., hepatocytomegaly, liver peliosis, cytoplasmic vacuolation, and hepatocellular degeneration/necrosis) are further evaluated with the BMDS modeling program for determination of a POD for the chronic p-RfD.

Inai et al. (1988) conducted a published, peer-reviewed study of the chronic effects of BHT in mice. B6C3F$_1$ mice were obtained from Charles River Japan at 4 weeks of age and observed for another 4 weeks prior to testing. Mice were housed in plastic cages, 5 males or 10 females per cage, in the same air-conditioned room. Male and female mice, 50/sex/group, were administered 0, 1, or 2% BHT (96% purity) in diet for 104 weeks, followed by a 16-week recovery period. Average daily doses reported by the study authors were 0, 1640, and 3480 mg/kg-day for males and 0, 1750, and 4130 mg/kg-day for females. However, the study authors note that incorporation of BHT into the food pellets was approximately 50% of the original content. Further data on this is not provided in the report, making assessment of the actual doses administered to each animal problematic. All animals were given feed and water ad libitum. The amount of food consumed and body weight were measured biweekly for the first 12 weeks and once every 4 weeks for the rest of the treatment period. At the end of the study, all of the surviving animals were sacrificed and necropsied. Animals that died prematurely also were necropsied. Tissues that were evaluated microscopically included the liver, lung, hematopoietic system, spleen, integumentary system, uterus, ovary, breast, pancreas, esophagus, forestomach, intestine, brain, pituitary gland, parathyroid gland, heart, and eyelid. Data on clinical chemistry were not reported. Organ weight is only provided for the liver. No information on GLP compliance is provided by the study authors.

Inai et al. (1988) reported a dose-dependent decrease in survival throughout the study in males but not in females. Dose-dependent decreases in average body weights were reported in the males and females (quantitative data not reported and graphical data could not be analyzed by the graph digitizer and thus data were not shown). Study authors reported increased absolute and relative liver weights in mice that did not develop liver tumors (see Table B.32). No changes in absolute or relative organ weights were seen in mice with hepatocellular tumors or in total mice groups. Hepatocellular lesions for male mice are shown in Table B.33. Dose-dependent increases in the number of foci of cellular alterations in hepatocytes and hepatocellular adenomas were seen in males but not females. The study authors also stated that nuclear pleomorphisms of hepatocytes in nontumorous areas were present but there were no hepatocellular necrosis, bile duct hyperplasia, or peliosis (data not reported). However, due to concerns regarding the accuracy of the reported doses, this study can only serve as supporting information, and a dose-response assessment is not appropriate. For these reasons, neither a NOAEL nor LOAEL can be derived from this study.

Hirose et al. (1986) published a peer-reviewed 16-week chronic study investigating the toxicity of BHT. The study authors administered a basal diet (5.1% fatty acid, 24.5% oleic acid, 48.5% linoleic acid, and 14.1% palmitic acid) containing 0 or 1% BHT (>98% purity) in powdered form to 26−32 (specific number not reported) 7-week-old male Syrian golden hamsters (weighing 85−115 g at study initiation) for 16 weeks. Adjusted daily doses were 0 and 183 mg/kg-day for males. The study authors recorded body weight weekly for the first 4 weeks and then monthly for the remainder of the study. The study authors did not provide data on food consumption throughout the study. In each treatment group, three hamsters were sacrificed at 1, 2, 3, and 4 weeks, and all of the remaining animals were sacrificed at 16 weeks for histological and autoradiographic examination. The histological examination included a complete postmortem examination and measurement of liver and kidney weight. No information on GLP compliance was provided by the study authors. No information on the statistical analysis performed by the study authors was presented although significant findings were listed in the results section. No raw data were provided by the study authors.

Hirose et al. (1986) noted that dietary administration of 1% BHT did not significantly affect body weight compared to control. Absolute liver weight was higher (although not significantly) from Day 3 to the conclusion of the study compared to the control. BHT administration did not significantly increase forestomach lesions or the labeling index (indicative of cell proliferation) in the forestomach in hamsters after BHT exposure.

Hirose et al. (1986) also administered 2-*tert*-BHA, 3-*tert*-BHA, and crude BHA in parallel to the BHT experiment using the same protocol. The study authors found increased incidence of labeled cells in the forestomach epithelium for animals exposed to BHA. The study authors discussed the possibility that the fatty acids in the basal diet may have become oxidized, allowing the test substance to attack the forestomach cells. They further suggested that BHT did not release enough free radicals to do significant damage as compared to BHA. Despite not identifying adverse effects of BHT administration, their finding presents an interesting explanation of interaction between the exposure compound and the components of the diet. Hirose et al. (1986) did not identify a NOAEL or a LOAEL in this study. The possibility of chemical interactions between the feed and the chemicals administered, together with the lack of raw data, preclude identification of a NOAEL or a LOAEL.

Deichmann et al. (1955e) exposed mongrel two dogs for 1 year via diet to average doses of BHT of 170, 280, 500, 470, 640, or 940 mg/kg-day (purity unreported) in ground beef. Dogs showed no signs of toxicity and unlike in the subacute study in dogs did not experience diarrhea. Histopathological analysis of several tissues (brain, pituitary, thyroid, thymus, heart, aorta, trachea, lungs, spleen, stomach, intestine, pancreas, liver, mesenteric lymph nodes, adrenal glands, kidneys, urinary bladder, uterus, ovaries, testes, epididymis, and prostate) did not show any pathologies related to BHT administration. The results presented in this review are for supporting purposes due to the lack of methodological details. Based on the lack of effects reported in the study a NOAEL of 940 mg/kg-day is identified. A LOAEL cannot be identified because no effects were reported at any of the administered doses.

Developmental and Reproductive Studies

McFarlane et al. (1997) published the results of a peer-reviewed range-finding study (McFarlane et al., 1997a) as well as a more extensive two-generation exposure study (McFarlane et al., 1997b). These studies were performed according to GLP compliance. The range-finding study exposed 2 male (200 g) and 16 female (60 g) F0-generation Wistar albino rats (Bantin and Kingman, Hull, U.K.) per dose group to doses of 0-, 500-, 750-, or 1000-mg/kg-day BHT (99.9 % purity) by diet and allowed them to cohabitate for 8 weeks. Except when paired together during mating, the males were housed individually in polycarbonate cages. Females were housed in groups of seven or eight, until pregnancy was confirmed, at which point they were housed singly. Standard breeding diet (CRM, Labsure, Manea, Cambridge, U.K.) and tap water were provided ad libitum. Environmental conditions were maintained at a temperature of 20 ± 3ºC, relative humidity of 30−70%, and a 12-hour light/12-hour dark cycle. Dosing at the same levels continued through pregnancy and lactation. Between Postnatal Days (PNDs) 6 and 10, litters were reduced or increased as needed to eight pups (F1), leaving a "maximum number of males," although the exact number was unspecified (McFarlane et al., 1997a). Dams and all but 14 pups per dose group were sacrificed at PND 21. The remaining pups (F1) were fed a control diet for the following 4 weeks.

Over the course of the study, all animals (F0 and F1) were weighed weekly and examined daily; Concentrations of BHT were adjusted in the feed biweekly, to maintain constant exposure, with the exception of pregnancy and lactation, when no dosing adjustments were made (McFarlane et al., 19997a). The liver, kidneys, adrenal, thyroid, spleen, pancreas, and lungs were taken from the sacrificed dams for examination. Study authors conducted autopsies on one pup per litter, and liver, kidneys and adrenals were removed for gross and microscopic examination. Study authors performed statistical analysis using the Student's *t*-test. Although the results of the histological exams are discussed in the study, no quantitative data is presented.

Treatment with BHT did not affect the body weights of F0 animals in any dose group prior to pregnancy (see Table B.34) (McFarlane et al., 1997a). Dams exposed to 750 and 1000 mg/kg-day gained less weight at the end of their pregnancies (89 and 80% of control, respectively) and did not show increased food consumption during lactation (which caused decreased body weights), in comparison to controls (data not provided). Dams in the 500-mg/kg-day dose group also lost body weight during lactation (96% of control) although this change did not reach the level of statistical significance. Mating success was not affected in any dose group. Examinations of the liver showed increased relative liver weights in animals in each dose group (151%, 162%, and 166% at 500, 750, and 1000 mg/kg-day, respectively). Histopathological analysis showed no effects in the liver of dams sacrificed at gestational days (GD) 19 or 20 (data not reported; methods describing which animals or how many were sacrificed at this time point were not reported). Although the quantitative data were not provided, study authors reported that dams exposed to 750 and 1000 mg/kg-day had hypertrophy of the centrilobular hepatocytes after lactation. Additionally, cytoplasmic vacuolization was noted in the enlarged cells as well as loss of glycogen. Dilation of the sinusoids in the centrilobular zone, bile duct proliferation (without evidence of inflammation or fibrosis), and proliferation of the endoplasmic reticulum (ER) of hepatocytes in liver sections were also reported. No other changes related to BHT exposure were observed in other tissues.

Table B.35 summarizes effects from exposure to BHT on the F1 pups, as reported by McFarlane et al. (1997a). No difference was observed in the number of pups born per litter to exposed dams compared to the control group. However, treated dams in the 500, 750, and 1000-mg/kg-day dose groups had reduced litter weights as compared to controls (96, 88, and 83%, respectively). Pup body weight at birth was only statistically significantly decreased in the 750-mg/kg-day group (91%, as compared to controls). The study authors observed normal postnatal development up to 1 week following birth but noted retarded development in the treated pups by the second week. Growth of pups in the 750-mg/kg-day and higher dose group before litter reduction was severely stunted (48 and 41%, as compared to controls, respectively). Reduction of the litters did not impact body weight reductions. Lethargy and poor fur also were observed in these animals. The weights of the pups in the 500-mg/kg-day dose group were decreased (66%, as compared to control). Absolute liver weights in pups in all dose groups were decreased, but when liver weights were compared to body weights, the relative liver weights were unchanged compared to controls. Notably, pups from smaller litters, where the study authors could not foster pups to make a litter of eight, were less affected.

McFarlane et al. (1997a) reported that pups maintained on a regular diet after weaning appeared healthy. Some (number not reported) of the 1000 mg/kg-day exposed pups suffered from weakness and were euthanized. Animals from this dose group that survived 1 week were healthy by 4 weeks past weaning. Weaned animals showed no gross abnormalities, no change in

relative liver weight, and no abnormal results after histopathological examination of the liver. Despite a lack of quantitative data, the results of histological analysis showed a lack of glycogen in animals exposed to 500 mg/kg-day or more. According to the study authors, the zona fasciculata had few lipid droplets as compared to control animals. No differences in the thyroids of exposed pups could be found as compared to controls. Based on the increased relative liver weights in the exposed dams, a maternal LOAEL of 500 mg/kg-day is identified. This effect is noted at the lowest dose administered in the study precluding identification of a maternal NOAEL. Based on the reduced body weights of F1 pups, LOAEL of 500 mg/kg-day is identified. This effect is noted at the lowest dose administered in the study precluding identification of a fetal NOAEL. The data for decreased body weight, increased relative liver weight in maternal dams, and decreased body weight of pups before reduction are further evaluated with the BMDS modeling program for determination of a POD for the chronic p-RfD.

The more extensive, two-generation exposure study (McFarlane et al., 1997b) was conducted using the same methods as described above for the range-finding study (no GLP information provided), with the exception that 7 male and 50 female rats per dose group were exposed to 0, 25, 100, or 500 mg/kg-day BHT. Animals were evaluated as described for the range-finding study with the following changes. Animals were allowed to cohabitate for 3 weeks after 5 weeks of exposure to BHT. Five dams from each dose group were sacrificed on GD 19 or 20 and analyzed as previously described. GD 19 or 20 fetuses were removed, sacrificed, weighed, and examined for abnormalities. Five of the fetuses were fixed for histological analysis, and five fetuses' livers were removed for ultrastructural examination or were pooled and homogenized for biochemical analysis. Five dams from each dose group were sacrificed at weaning (PND 21) as were their litters and all F0 males. The livers were removed from these dams for histological analysis. Liver, kidneys, adrenal glands, and thyroid were removed from at least four of the pups per dam for histological analysis. Additionally, livers from five control and 500 mg/kg-day treated pregnant and nonpregnant females were removed for microscopic and biochemical analysis. Sixty pups per dose group were sacrificed at 4 and 22 weeks after weaning from all dose groups. Biochemical analysis included: glucose-6-phosphatase activity, cytosolic glutathione-*S*-transferase, total glutathione, 7-ethoxyresorufin *O*-deethylase, benzphetamine *N*-demethylase, pentoxyresorufin *O*-dependentylase, epoxide hydrolase, total microsomal cytochrome p-450 and homogenate, and cytosolic and microsomal protein levels.

Similar to the range-finding study (McFarlane et al., 1997a), no significant differences in mating success, weight gain, or food consumption were seen prior to lactation in F0 rats exposed to 25, 100, or 500 mg/kg-day BHT (McFarlane et al., 1997b). Table B.36 shows that relative liver weight was increased in the 500 mg/kg-day dams sacrificed on GD 19 or 20 (106% as compared to control) but did not reach the level of significance. The relative liver weights of lactating and the time-control nonlactating dams were increased 135% for both groups compared to their respective control (see Table B.37). Lactating females (PND 21) were reported to have the same hypertrophy of the liver that was observed in the range-finding study (data not reported) (McFarlane et al., 1997a). Although no quantitative data were presented, livers of dams after lactation showed dose-dependent centrilobular cell enlargement at the 100- and 500-mg/kg-day but not at 25 mg/kg-day. According to the study authors, no other organs were affected by treatment, except the thyroid, where hyperactivity in the 100- and 500-mg/kg-day dose groups was observed. Microscopic examination of livers from dams exposed to 500 mg/kg-day showed a dose-dependent proliferation of smooth ER. Dams sacrificed on

PND 21 had a dose-related reduction in fat surrounding the body wall, kidneys, and adrenal glands.

Biochemical analysis conducted by McFarlane et al. (1997b), summarized in Table B.37, showed that glucose-6-phosphatase levels were lower in lactating and nonlactating rats exposed to 500 mg/kg-day than those in controls (76% and 47%, respectively). Total glutathione in 500 mg/kg-day lactating rats was significantly decreased as compared to lactating control (39%), but increased in nonlactating 500 mg/kg-day rats as compared to control (110%). Nonlactating and lactating rats exposed to 500 mg/kg-day BHT had increased total cytochrome p-450 levels (165% and 169%, respectively) and glutathione-*S*-transferase levels (215% and 294%, respectively), with the greatest increases seen in the lactating rats. Nonlactating and lactating rats exposed to 500 mg/kg-day had significantly up-regulated pentoxyresorufin *O*-deethylase (6462% and 8449%, respectively) and slightly down-regulated (not statistically significant) ethoxyresorufin *O*-deethylase (96% and 83%, respectively).

McFarlane et al. (1997b) reported no effects on litter number of dams sacrificed at GD 19 or 20 (see Table B.38). Body weight of fetuses also were statistically and biologically decreased in the second highest dose group. In the highest dose group, decreased fetal body weight was not statistically changed but was still biologically significantly (>5% change considered to be biologically significant in non-adult pups) decreased. Relative liver weight was statistically and biologically significantly increased at the highest dose tested. Histopathological and biochemical examination of livers from treated fetuses showed no changes compared to controls. Biochemical analysis of these fetuses did not show changes in liver enzyme levels (glutathione *S*-transferase, total glutathione, 7-ethoxyresorufin *O*-deethylase, benzphetamine *N*-demethylase, and epoxide hydrolase) compared to controls, with the exception of a decrease in glucose-6-phosphatase (58% of control) at the 100 mg/kg-day dose-level. Pups born to dams receiving 500 mg/kg-day of BHT during gestation and lactation and then fed diets providing 250 mg/kg-day after weaning, examined at weaning (PND 21) had an increased relative liver weight (125%) compared to controls (see Table B.39). Biochemical analysis of these treated pups (PND 21) revealed increased levels of glutathione *S*-transferase, 7-ethoxyresorufin *O*-deethylase, benzphetamine *N*-demethylase, and epoxide hydrolase in the pups exposed to 250 mg/kg-day, as well as increased levels of 7-ethoxyresorufin *O*-deethylase, benzphetamine *N*-demethylase, and epoxide hydrolase in the 100-mg/kg-day group. No change was seen in the total glutathione levels, but dose-related, statistically nonsignificant decreases in glucose-6-phosphatase were seen in the 100- and 250-mg/kg-day dose groups (82% and 77%, respectively) (see Table B.39).

McFarlane et al. (1997b) noted an increase in relative liver weights in pups born to 500 mg/kg-day dams, fed 250 mg/kg-day after weaning, and sacrificed at 4 and 22 weeks after weaning (111% and 107%, respectively), and body weights decreased (93% and 87%) (see Tables B.40 and B.41). No liver histopathology findings were reported, aside from a slight dilation of the sinusoids in exposed animals. No changes were observed in other organs of the 100- and 250-mg/kg-day dose groups except for mild hyperactivity of the thyroid and hypertrophy of the zona fasciculata cells of the adrenal glands. Proliferation of the smooth ER was reported in the livers of pups exposed to 250 mg/kg-day, which the study authors state is supported by observed centrilobular eosinophilia and up-regulation of cytochrome p-450 (124%, only at 22 weeks). Dilation of the sinusoids and loss of glycogen also were observed. Other observations included large vacuoles in the hepatocytes and osmiophilic material in the lumen of

the bile canaliculi. Biochemical analysis of treated pups revealed no significant changes in the levels of benzphetamine *N*-demethylase at 4 and 22 weeks postweaning (see Tables B.40 and B.41). Ethoxyresorufin *O*-deethylase was up-regulated at 4 weeks postweaning in all dose groups (25 mg/kg-day: 146%, 100 mg/kg-day: 138%, 250 mg/kg-day: 147%), but at 22 weeks after weaning the increase was statistically significant only in the animals exposed to 100 mg/kg-day (140%). Glutathione *S*-transferase and epoxide hydrolase were significantly increased in the 250-mg/kg-day dose group at 4 weeks (158%) and 22 weeks (146%) after weaning and in the 100-mg/kg-day group at 4 weeks (143%) postweaning. Total glutathione was decreased in the 250 mg/kg-day-treated pups at 4 weeks (73%) and 22 weeks (63%) after weaning while both the 100 and 250 mg/kg-day offspring animals had glucose 6-phosphatase reduced to 72−80% of the control values during both study periods (see Tables B.40 and B.41).

The study authors conclude that for both the range-finding (McFarlane et al., 1997a) and the main study (McFarlane et al., 1997b) administration of BHT had no systemic effect on treated animals. There is no evidence of exposure influencing mating success or fetal development. Dams given doses of 500 mg/kg-day and greater had pups that did not gain as much weight between nursing and weaning of pups, as compared to controls, and this trend continued after being given the control feed. The study authors suggested that this weight retardation is due to malnutrition, rather than as a result of BHT exposure because pups in reduced litter sizes in these exposure groups had a less severe stunting of their weight (data not reported). Pups exposed to 100 and 250 mg/kg-day reportedly had mild hyperactive thyroid and hypertrophy of the zona fasciculata cells in the adrenal glands. Pups of the dams given doses of 500 mg/kg-day and greater had upregulated liver xenobiotic-metabolizing enzymes, increased relative liver weights after weaning, and hepatocyte abnormalities (proliferation of the smooth ER and eosinophilia). Liver changes, including vacuolization and proliferation of the ER were also clearly seen in the lactating dams treated with 500 mg/kg-day or more, which was supported by the observed decrease in glucose-6-phosphatase (a possible indicator of liver damage). The study authors reported that BHT in combination with lactation exacerbated the stress on the liver, reducing the nutrient content available to the pups. Lactation also reportedly increased food consumption and thus the exposure levels of the dams.

Based on the liver effects observed in dams, as well as females exposed during mating, gestation, and lactation (estimated 14 weeks), a maternal LOAEL of 500 mg/kg-day is identified along with a NOAEL of 100 mg/kg-day. Based on a biological (≥5% change considered to be adverse for fetal effects) and statistical significant decrease in body weight observed in the F1 generation, a LOAEL of 100 mg/kg-day is identified from this study, with a corresponding NOAEL of 25 mg/kg-day.

Price (1994) conducted a study using F1 Wistar rats generated from the study by McFarlane et al. (1997). The original report was not obtainable; limited information was cited in the OECD SIDS (2002) report. This study was performed according to GLP guidelines. Male F1 rats (approximately 60/dose group) were administered BHT (99.9 % purity) in the diet for 22 months postweaning with interim sacrifices at 1, 6, 11, and 16 months. As discussed in the OECD SIDS (2002) report, doses are 0, 25, 100, or 250 mg/kg-day. The study authors reported decreased body weight in the mid- and high-dose groups throughout the 22-month treatment duration. The following liver effects were observed: increased relative liver weight and altered hepatic nodules at 16 months, enlarged and eosinophilic centrilobular hepatocytes at 6 months, and periportal induction of gamma-glutamyl transferase at 11 months. Immunocytochemistry

was performed for only the high-dose group and revealed increased altered hepatic foci cytochrome P450 1B at 16 months and total cytochrome P450 at 11, 16, and 22 months. Microscopic examination of the thyroid revealed hyperactivity in the mid- and high-dose groups at 11 months. Chronic progressive nephropathy was observed in all rats including controls at 11 months. No effects on the adrenals were observed. The study authors reported that no tumors were observed. The OECD SIDS (2002) report identified a NOAEL of 25 mg/kg-day based on liver, kidney, thyroid and adrenal effects.

Tanaka et al. (1993) published a peer-reviewed, three-generation dietary exposure study in which they exposed male and female Crj:CD-1 mice (weights not reported) to BHT (purity not stated). Mating occurred at 9 weeks of age, and rearing was consistent across all three generations. One hundred male and female four-week-old Crj:CD-1 mice were acquired from Charles River Japan Inc., Kanagawa, Japan. Mice were housed individually in polycarbonate solid-floored cages with wood flakes at 24 ± 1°C and 55 ± 5% humidity. Of these, 10 male and 10 female mice and their subsequent offspring from mated nonsiblings served as controls. Ten male and ten females at 5 weeks of age were fed diets containing 0.015% (150 ppm), 0.045% (450 ppm), 0.135% (1350 ppm), or 0.405% (4050 ppm) BHT (purity not reported). Appropriate body-weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for different strains of mice by EPA (1994b) for body weight (0.024725 kg for both sexes) and food consumption (0.004825 kg/day for both sexes) are used in the dosimetric calculation. Corresponding adjusted daily doses are 29, 88, 263, and 790 mg/kg-day for both males and females. At 9 weeks of age, F0 females and F0 males in the same dose group were allowed to cohabitate for 5 days. F1 pups were weaned at 4 weeks of age and mated at 9 weeks of age, while avoiding sibling matings to produce an F2 generation. This study's conformance with GLP guidelines could not be determined.

After birth, on PND 0, Tanaka et al. (1993) recorded litter size, litter weight, and sex ratio (male/female) of the F1 generation. No characteristics of the dams at birth or during gestation were reported. F1 pups were evaluated for functional and behavioral endpoints including: surface righting and negative geotaxis on PNDs 4 and 7, cliff avoidance on PND 7, swimming behavior (direction, head angle, and limb movement) on PNDs 4 and 14, and olfactory orientation on PND 14. Field activity was assessed at 3 weeks of age (PND 21) for 3 minutes by observing ambulation, rearing, 180° turn, defecation, urination, and preening. Litter size, litter weight, and body weight were analyzed statistically using Bonferroni's multiple comparison test following an ANOVA or Kruskal-Wallis test. A chi-square test was used to analyze the sex ratio. Neurobehavioral parameters were analyzed with the Mann-Whitney *U*-test. All endpoints were consistently measured between different generations.

Tanaka et al. (1993) reported no dose-related effects of BHT on number of litters, litter size, litter weight at birth, or sex ratio in the F1 or F2 generations (see Table B.42). The low-dose group's body weights increased statistically significantly compared to control in both generations, whereas, in the first generation, pup birth weights decreased in the two highest-dose groups (quantitative data not reported and thus data not shown here). This effect was not observed in the F2 generation. Neurobehavioral effects in F1 and F2 pups were also inconsistent across doses (see Tables B.43 and Table B.44). All exposed F2 males turned 180° significantly less than controls (50%, 58%, 52%, and 45%, in the 29, 88, 263, and 790-mg/kg-day groups, respectively) (see Table B.44). Negative geotaxis in the highest-dose group of F1 males on PND 4 was significantly greater than controls. All other neurobehavioral effects that were

significantly different did not increase with increasing dose. Among these, only F2 male pups at PND 4 had any other neurobehavioral effects present at higher doses. The most significant neurobehavioral effects therefore were observed in F2 males (see Tables B.43 and Table B.44).

The dose levels of BHT resulted in no dose-related effects on neurobehavioral or reproductive endpoints, except for a statistically significant decrease in the 180º turn test in F2 males that was not observed in the F2 females. Based on this effect alone the results are not significantly adverse to warrant assigning a LOAEL to this compound. A $NOAEL_{ADJ}$ of 790 mg/kg-day is identified.

The Tokyo Metropolitan Research Laboratory of Public Health (1992b) sponsored an unpublished teratogenicity study in JCL-ICR mice with BHT (purity not reported) in olive oil that was not peer-reviewed. Two types of testing were conducted: a repeat-dose and a single-dose administration. The study authors did not state whether the study protocol adhered to GLP standards. In the repeated-dose test, BHT was administered by gavage once per day for 7 days to pregnant mice (n = 26−30) on GDs 7−13 at dose levels of 70, 240, or 800 mg/kg-day, including both negative controls in which no substance was administered and vehicle controls which were administered olive oil. In the single-dose test, BHT was administered by gavage to pregnant female mice (n = 19−20) on GD 9 at dose levels of 1200 or 1800 mg/kg-day, including a negative control group. Study authors measured body weights and observed general health of the animals daily. At GD 18, dams were sacrificed, and investigators counted the number of implantation sites, corpus luteum absorbed embryos, and dead and live fetuses. Also at necropsy, study authors measured organ weights and recorded gross observations of toxicity in the dams. In the surviving fetuses, investigators measured body weights and major organ weights, calculated the sex ratio, and examined embryos for external malformations. Study authors also randomly selected five dams (dose group not specified) to examine the internal and skeletal abnormalities of the live fetuses.

In the 7-day repeat-dose test, the Tokyo Metropolitan Research Laboratory of Public Health (1992b) did not observe any significant changes in body weight or behavior in dams treated at any dose level of BHT versus the vehicle or negative controls. The mean spleen and kidney weights of dams in the high-dose (800-mg/kg-day) group were significantly different from control (no further data provided; data table in study report is illegible). There were no significant differences in gestation rate, numbers of corpus luteum and implantations, dead and live fetuses, and sex ratio between any of the treatment groups versus controls. Although body weights were significantly lower in male fetuses of dams treated with BHT, no dose-response trend was observed, and study authors did not consider this finding to be biologically significant. Investigators also reported significantly lower incidence of accessory sternebrae (1.4%) and a significantly higher incidence (11.1%) of cervical ribs in the high-dose (800-mg/kg-day) group versus controls, but these differences were not considered treatment related when compared to background incidences. Study authors did not consider the observations of cleft palate, eyelid opening, and polydactyl in the BHT-treated fetuses to be treatment-related due to similar incidences observed in the control groups. No internal abnormalities or differences in ossification rates were observed in any of the fetuses in the BHT-treated groups.

In the single-dose test, dams in the 1200- and 1800-mg/kg-day groups experienced 10% (2/20) and 25% (5/20) mortality, respectively (Tokyo Metropolitan Research Laboratory of Public Health, 1992b). The mean spleen weight of the dams in the 1200-mg/kg-day dose group

and the mean spleen and lung weights of the dams in the 1800-mg/kg-day dose group were significantly higher than the negative control group (no further data provided; data table in study report is illegible). One dam in the 1200-mg/kg-day dose group had no surviving fetuses; all died at an early stage. Though the 1800-mg/kg-day dose group did not have a similar occurrence and the incidence of dead fetuses in this group was similar to controls, the study authors did not consider the effect in the 1200-mg/kg-day dose group to be biologically significant. There were no significant differences in gestation rate, numbers of corpus luteum and implantations, dead and live fetuses, and sex ratio between any of the treatment groups compared to controls. Investigators observed no significant differences in external, internal, or skeletal abnormalities in the live embryos of BHT-treated dams versus controls. Study authors did not report a NOAEL or LOAEL for either experiment. As mentioned previously, the data tables are largely illegible making these studies unusable for derivation of a subchronic p-RfD.

Olsen et al. (1986) is selected as the principal study for deriving the subchronic and chronic p-RfDs and the p-OSF. Olsen et al. (1986) published a peer-reviewed developmental, reproductive, and carcinogenicity study in rats exposed in utero to BHT. The study authors reported both subchronic and chronic noncancer effects as well as chronic cancer effects. Groups of male and female specific-pathogen-free (SPF) 7-week-old F0 Wistar rats (body weights unreported) were treated with 0, 25, 100, or 500 mg/kg-day BHT (>99.5% purity) in a semisynthetic diet (n = 60, 40, 40, and 60, respectively, for each dose group) confirmed to be free of aflatoxin and nitrosamines. After 13 weeks of exposure, 40, 29, 30, and 44 litters from each dose group were used to populate exposure groups (0, 25, 100, and 250 mg/kg-day) with 100, 80, 80, or 100 male and female rats, respectively. F0 animals were excluded from the study after mating for the males and lactation for the females. Because F0 female rats treated with 500 mg/kg-day BHT exhibited an adverse effect in the liver, the F1-generation rats were administered a high dose of 250 mg/kg-day instead of 500 mg/kg-day. After weaning, F1 animals were exposed to BHT in a semisynthetic diet until 141−144 weeks of age. No information was provided regarding GLP compliance.

Olsen et al. (1986) recorded the rats' body weights on a weekly basis until the animals were 31 weeks of age; afterward, they recorded body weights once every 2 weeks. Food consumption was recorded every week. Hematocrit and hemoglobin levels were determined in whole blood and in red and white blood cells using blood collected from 20 F1 male and female rats after 9, 19, 43, and 108 weeks of BHT treatment. Serum was collected and analyzed for glucose, free and total cholesterol, triglycerides, blood urea nitrogen (BUN), and phospholipid levels. All F1 rats were inspected on a regular basis (time frame not specified) for the presence of tumors. At study termination, all surviving animals (141−144 weeks of age) were sacrificed. A gross necropsy was performed on animals that were sacrificed at study termination and animals that died during the course of the experiment. Liver, kidneys, lungs, brain, heart, spleen, pituitary gland, thymus, pancreas, thyroid, adrenal glands, testes, ovaries, uterus, seminal glands, mesenteric and axillary lymph nodes, salivary glands, urinary bladder, gastrointestinal tract, spinal cord, skeletal muscle, peripheral nerve, bone, skin, mammary gland, eyes, and the Harderian gland were preserved for further examination. Animals that developed the first tumors beyond 43 weeks were included in the total effective number of animals. Biochemical, hematological, and other biological data were statistically analyzed using the Student's t-test, while the Armitage-Cochran test for linear trend was used to determine preweaning mortality in various litters. Mortality and tumor incidence were analyzed by the Peto method (a comparison of observed and expected values).

Olsen et al. (1986) reported that F0 rats treated with 25, 100, or 500 mg/kg-day BHT did not exhibit any differences in food consumptions rates when compared to the concurrent control group. The study authors reported a statistically significant decrease in body weight compared to the controls in male and female F0 rats treated with 500 mg/kg-day BHT (data not reported). This decrease was noted beginning in Week 6 of the treatment, and it persisted throughout their lifetimes. The study authors reported that the number of litters with 10 or more pups decreased in a dose-dependent manner, with dams treated with 500 mg/kg-day BHT exhibiting a statistically significant decrease in the number of litters, as indicated by the Cochran-Armitage statistical analysis. Pup viability was not affected as a result of exposure to BHT. Average F1 pup birth weights in the 100- and 500-mg/kg-day dose groups were slightly lower (97%) compared with the concurrent control groups (see Table B.21). At weaning, F1 pups exposed to BHT exhibited significant and dose-dependent depression in body-weight gain compared with the corresponding control group (95% at 25 mg/kg-day, 94% at 100, and 60% at 500 mg/kg-day).

F1 rats did not exhibit any change in food consumption rates as a result of exposure to BHT (Olsen et al., 1986). A dose-dependent and significant depression in mean body weight was noted in animals treated with BHT compared to the concurrent control group (see Table B.22). For example, at 138 weeks, male body weights were 92, 89, and 85% and female body weights were 100, 97, and 90%, for the 25, 100, and 250 mg/kg-day doses, respectively. The body weight changes at 100 and 250 mg/kg-day in males and at 250 mg/kg-day in females are considered to be biologically significant based on a BMR of 10% for changes in adult animals. Mortality was reported to be higher in F1 control groups compared with the BHT-treated groups (see Table B.23). At 104 weeks, 72% of males and 86% of female F1 rats treated with 250 mg/kg-day BHT survived compared to 70% of males and 69% of females in the control group. At study termination, 44 and 39% of male and female F1 rats survived in the 250-mg/kg-day dose group compared to 16 and 17% in the male and female control groups, respectively (see Table B.23). The study authors reported that there was a significant difference in longevity in both sexes. The higher mortality of control F1 male rats compared with BHT-treated male F1 rats was mainly attributed to inflammation of the bladder that was often associated with kidney stones. In female F1 rats, higher mortality of controls compared with the BHT-treated groups was primarily due to the occurrence of nephropathy and tumors in the pituitary gland. Exposure to BHT had no effect on clinical appearance or animal behavior. However, a slight reddish discoloration of the urine was noted in male F1 rats exposed to 250 mg/kg-day BHT. Hematological analysis indicated no treatment-related changes in the F1 rats. Serum chemistry analysis exhibited elevated cholesterol and phospholipid levels during the first year of exposure in female F1 rats treated with 250 mg/kg-day BHT compared with the concurrent control group (see Table B.24). Triglyceride levels were statistically significantly lower in both male and female F1 rats in the BHT-treated groups compared with the corresponding controls during Weeks 19, 43, and 108 (see Table B.24).

Olsen et al. (1986) reported that incidences of hepatocellular adenomas and carcinomas in F1 males and hepatocellular adenomas in F1 females were elevated in BHT-treated animals compared to the corresponding control groups (see Table B.25). In the 0, 25, 100, and 250-mg/kg-day dose groups, the percent incidences of hepatocellular adenomas were 1, 1, 6, and 18% in male rats and 2, 4, 8, and 12% in female rats, respectively. The respective percent incidences of hepatocellular carcinoma were 1, 0, 1, and 8% in males and 0, 0, 0, and 2% in females. The first carcinoma in BHT-treated F1 rats was observed during Week 132 in one male

treated with 250 mg/kg-day BHT. The rest of the carcinomas were observed at study termination. Only one control F1 male exhibited carcinoma at 117 weeks of age. The first adenoma was observed in one male treated with 250 mg/kg-day BHT after 115 weeks, but most adenomas were reported in both sexes at study termination (Weeks 141−144; Table B.26). There was no intralitter correlation among rats diagnosed with hepatocellular tumors. Gross examination of hepatocellular adenomas indicated that the adenomas were 4−30 mm in diameter, whereas the carcinomas were 15 mm or more in diameter. Neoplasms were not preferentially located in the liver. Occasional ascites in connection with large carcinomas also were observed. Although basophilic adenomas were observed on occasion, eosinophilic adenomas were observed more frequently. Hepatocellular carcinomas were comprised of basophilic hepatocytes that formed a trabecular pattern, and, in some carcinomas, a projection of irregular cords without endothelial lining was seen in dilated sinusoids. The study authors reported that metastases of the carcinomas were not observed. The incidence of hepatocellular adenomas was high in both the male and female F1 rats in the 250-mg/kg-day dose group, but a higher number of hepatocelluar carcinomas was observed in the F1 males compared with the F1 females (see Table B.25), indicating that the males were more susceptible to BHT than females.

Besides hepatocellular adenomas and carcinomas, the following tumors were observed by Olsen et al. (1986) in either males or females or in both sexes: thyroid C-cell adenoma (females), thyroid C-cell carcinoma (males), islet-cell adenoma in both males and females, exocrine adenomas of the pancreas and haemangioma and reticulum-cell sarcoma of the reticuloendothelial system in males, theca granulosa-cell adenoma in females, adenoma and adenocarcinoma of the uterus and thymoma in females, and ductular adenoma of the mammary gland in females. However, the incidence of these tumors was not statistically significantly higher when compared to the corresponding control group. The low incidence of theca granulosa-cell adenomas in the F1 females was significantly higher in the trend analysis but only in the 250-mg/kg-day dose group. The study authors reported that overall the number of F1 male and female rats exhibiting malignant tumors or multiple tumors, excluding hepatocellular tumors, was slightly—but not significantly—higher in the 250-mg/kg-day dose group compared with the concurrent control group (see Table B.27).

The study authors also reported the occurrence of nonneoplastic lesions in the liver including a dose-dependent increase in the incidence of bile duct proliferation and cysts in F1 males, and focal cellular enlargement in F1 females (see Table B.28). Heart nephropathy and fibrosis also were noted to occur less frequently in rats treated with BHT than in corresponding controls. Other nonneoplastic lesions that were observed occurred on an incidental basis and according to study authors are not related to BHT exposure.

After utilizing tests of heterogeneity using the trend analysis, Olsen et al. (1986) concluded that the number of hepatocellular adenomas and carcinomas was increased significantly in F1 male rats, but, in F1 females, only the hepatocellular adenomas were increased significantly (see Table B.25). A dose-related increase in total hepatocellular tumors (sum of adenomas and carcinomas) was determined in both sexes and was statistically significant at the highest dose tested. Hepatocellular tumors were detected when F1 rats were at least 2 years of age (see Table B.26). Tumors observed in other organs had a low incidence and were not statistically significantly different from the corresponding control group. Maternal $LOAEL_{ADJ}$ of 500 mg/kg-day, and a corresponding $NOAEL_{ADJ}$ of 100 mg/kg-days are identified based on reported decreases in weight in the dams. Because study authors reported both

subchronic and chronic effects for F1 adult rats, a NOAEL and LOAEL can be identified for both study durations. For subchronic effects, a $LOAEL_{ADJ}$ of 250 mg/kg-day and a corresponding $NOAEL_{ADJ}$ of 100 mg/kg-day are identified based on decreased body weight. For chronic effects, a $LOAEL_{ADJ}$ of 100 mg/kg-day and a corresponding $NOAEL_{ADJ}$ of 25 mg/kg-day are identified based on chronic body-weight depression.

Other Studies

No other quantitative data were located regarding the toxicity of BHT to animals following other exposures.

Inhalation Exposures

No quantitative data were located regarding the toxicity of BHT to animals following subchronic or chronic inhalation exposure.

Other Exposures

No quantitative data were located regarding the toxicity of BHT to animals following other exposures.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Table 3 summarizes studies examining the genotoxicity and neurotoxicity of BHT, as well as its effects on the transcriptome, metabolism, and toxicokinetics. Toxicokinetic and transcriptome analysis are discussed in greater detail due their importance in supporting the provisional toxicity values. Details of the neurotoxicity study are also presented because of the toxicological significance of neurological effects. A summary of the genotoxicity results is provided because BHT has been shown to be negative for genotoxic activity, and thus these results do not affect the derivation of the provisional toxicity values, or any associated mode of action.

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Neurotoxicity	(unreported number and sex) F1 Swiss Webster mouse, gestation, lactation and 0.5% (5000 ppm) diet exposure GD1−6 weeks.	Exposure decreased sleeping and increased fighting as compared to control. Exposed animals did not exhibit learned behaviors in the automated avoidance conditioning climbing screening. Males showed significant increases in isolation-induced aggression when compared to control.	Due to exposure pre- and postnatally to BHT, it was not possible to determine which stage caused the adverse effects. The study authors believed that the change in aggressive behavior was developmental due to the results of the acute testing. The study authors further noted that BHT may be largely lipophilic in nature and therefore interferes with the synaptic vesicle membrane.	Stokes and Scudder (1974)
Transciptome analysis	Male Sprague-Dawley rat, diet (0, 28, 88, 167, 321, or 1159 mg/kg-day), 28 days.	Hepatic mRNA levels of the phase 1 cytochrome P450 isozymes, CYP2B1/2, increased. BHT also increased the mRNA levels of GST 1 among the phase 2 metabolizing enzymes in a dose-dependent manner.	Indicates potential role of liver enzyme induction in the metabolism and liver effects seen after exposure.	Stierum et al. (2008)
Toxicokinetic	Male and female DDY/Slc mice and male Sprague-Dawley rat given single doses labeled with ^{14}C ([^{14}C])BHT of 20 or 500 mg/kg by stomach intubation.	Concentrations were highest in the stomach, intestines, gall bladder, and urinary bladder. Excretion over 7 days was 41−65%, in the urine at 26−50%, and in expired air at 6−9%. Forty-three metabolites were identified in the urine and feces of rats and mice. All traces of BHT or metabolites were completely excreted by 7 days.	BHT is highly metabolized, and is absorbed primarily into part of the digestive and urinary system.	Matsuo et al. (1984)
Toxicokinetic	White male Wistar rats were given doses of [^{14}C]-labeled BHT (or its related compounds) via intravenous (i.v.) or intraperitoneal (i.p.) injection.	Intravenous: 46% of administered BHT was excreted in the bile in the first hour and 94% was excreted in the bile 6 hours after dosing. Intraperitoneal: 32% excreted in the bile 2 hours following dosing, and 52% excreted 6 hours after dosing.	BHT and related metabolites are rapidly metabolized and excreted in bile. The differences between the i.v. and i.p. administrations suggest differences in the kinetics, potentially in the peritoneum, which may influence the metabolism of BHT.	Holder et al. (1970)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	Salmonella strains TA98, TA100, and TA1537 were exposed to 0.05, 0.5, 5, 50, or 500 µg/plate BHT in an Ames assay. Colonies were evaluated for mutagenic effects with and without metabolic activation by S9 liver fraction. Positive responses were characterized by a 50% increase in the number of revertant colonies over the spontaneous frequency.	Study authors reported negative results for all strains at all dose levels with and without S9 activation.	These results suggest that BHT is not mutagenic in Salmonella strains TA98, TA100, or TA1537.	Bruce and Heddle (1979)
Genotoxicity	Male *Drosophila melanogaster* were injected with 0.02µl of 0.001% or 0.0001% BHT and mated. Female offspring were evaluated for sex-linked recessive lethal mutations and males for chromosome translocations. Some males were also exposed to 2.4 krads gamma rays and evaluated for radiosensitizing effects.	Study authors found no mutagenic effects from BHT exposure alone, but reported extreme radiosensitizing effects of gamma ray-induced sex-linked recessive lethal mutations and chromosome translocations.	Study authors concluded that BHT alone does not cause sex-linked recessive lethals or chromosome translocations in mature sperm of Drosophila melanogaster, but that BHT is an effective radiosensitizer of these mutations.	Kamra (1973)
Genotoxicity	Male *Drosophila melanogaster* were either injected with 0.4µl of a 0.05% solution BHT in saline ethanol or fed a 0.2% solution BHT and mated. Offspring were evaluated for II-III translocations and sex-linked recessive lethals. Offspring of males that were also treated with 2krads of X-rays were also evaluated for dominant lethals.	Study authors found that BHT treatment alone caused no increased frequency of translocations or sex-linked recessive lethals. Treatment with BHT as well as irradiation did not increase the frequency of II-III translocations, or dominant lethals, however, study authors reported a decrease in sex-linked recessive lethals.	These results suggest that BHT does not increase radiation-induced genetic damage in *Drosophila melanogaster*.	Barnett and Muñoz (1980)
Genotoxicity	Salmonella strains TA98, TA100, TA1537, and TA1538 were exposed to BHT in concentrations below 10 µmol/plate (according to a toxicity determination assay) as well as known mutagens in an Ames assay to determine the inhibitory effects of BHT on mutagenic effects of these chemicals.	Study authors reported that BHT reduced inversions induced by every mutagen requiring S9 activation, but did not reduce inversions induced by direct-acting chemicals.	Study authors concluded that in nontoxic concentrations, BHT inhibits an enzymatic activation mechanism of promutagens, reducing inversions in salmonella strains TA98, TA100, TA1537, and TA1538.	McKee and Tometsko (1979)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	Chinese hamster V79 cells were exposed to BHT along with benzo(a)pyrene and treated with mouse liver S9 fraction to evaluate the inhibitory effects of BHT on benzo(a)pyrene-induced mutations.	Study authors reported fewer mutations in cultures exposed to both BHT and benzo(a)pyrene than in cultures exposed to benzo(a)pyrene alone. At a ratio of 1:1 benzo(a)pyrene to BHT, there was no significant difference between mutant frequencies and those of spontaneous background level.	These results suggest that BHT has an inhibitory effect on benzo(a)pyrene-induced mutations.	Paschin and Bahitova (1984)
Genotoxicity	Male *Drosophila melanogaster* were injected with 0.001% BHT and exposed to 0, 1.2, 2.4, or 3.6 krads gamma-rays and mated. Generation F1 was evaluated for X^B chromosome loss and sex ratio and the F2 generation for sex-linked recessive lethal mutations.	Study authors reported increased frequency of X^B chromosome loss in generation F1 and of sex-linked recessive lethals in F2, however, these effects were not significant. Study authors attributed this increase in sex-linked recessive lethals to radiosensitization effects of BHT.	These results suggest that BHT enhances the mutagenic effects of gamma-rays in *Drosophila melanogaster*.	Prasad and Kamra (1974)
Genotoxicity	Blood leukocyte cultures drawn from a male human were exposed to 7,12-dimethylbenzanthracene along with 0 or 0.21 µmol BHT and evaluated for chromosomal aberrations.	Study authors reported a 63.8% reduction in the number of chromosome breaks in cultures exposed to dimethylbenzanthracene as well as BHT.	These results suggest that BHT has an inhibitory effect on 7,12-dimethylbenz(α)anthracene- induced chromosomal breakage.	Shamberger et al. (1973)
Genotoxicity	Salmonella strains TA98 and TA100 were exposed to 0−50 µg/plate BHT and evaluated for mutagenic effects with and without Aroclor 1254-induced rat liver S9 fraction. Strains also were exposed to BHT in conjunction with aflatoxin B_1 and evaluated for effects on mutagenicity.	Study authors reported no mutagenic effects of BHT alone in either strain with or without metabolic activation, but reported toxic effects at doses of 20 µg/plate and higher. A two-fold increase in revertant colonies was reported in both strains with the addition of 5−20 µg/plate BHT to aflatoxin B_1.	These results suggest that the presence of BHT enhances the mutagenic effects of aflatoxin B_1.	Shelef and Chin (1980)
Genotoxicity	Male Sprague-Dawley rats were fed a diet of 0.4% BHT for 10 weeks and mated. Females were sacrificed and evaluated for presence of dominant lethals by the number of live and dead implants.	Study authors reported weak but statistically significant increases in the frequency of dominant lethal mutations.	These results suggest that BHT causes dominant lethal mutations in Sprague-Dawley rats.	Sheu et al. (1988)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	Male hybrid (101 × C3H) mice were fed a diet of 1% BHT for 8 weeks and mated with hybrid (SEC × C57BL and C3H × C57BL) female mice. Females were evaluated for presence of dominant lethals and offspring were evaluated for presence of translocations.	Study authors reported no evidence of a dominant lethal effects or induction of heritable translocations.	These results suggest no mutagenicity of BHT in hybrid mice.	Sheu et al. (1988)
Genotoxicity	*Salmonella typhimurium* strain TA98 was exposed to 0, 10, 50, 100, 500, 1000, or 5000 µg/plate BHT with and without promutagens benzo(a)pyrene and 2-aminoanthracene. Colonies were evaluated for mutagenic effects with Aroclor-induced rat liver S9 fraction.	Study authors found no mutagenic effects of BHT with metabolic activation. No effects of the presence of BHT on benzo(a)pyrene were reported, however, a significant increase in the number of revertant colonies was reported in cultures treated with 2-aminoanthracene and 10 µg BHT and higher.	These results suggest that BHT has an enhancing effect on the mutagenicity of 2-aminoanthracene in salmonella strain TA98.	Dertinger et al. (1993)
Genotoxicity	Male ICR/Ha Swiss mice were injected (i.p.) with 250, 500, 1000, or 2000 mg/kg-body weight BHT and subsequently mated with females. Females were sacrificed and evaluated for dominant lethal mutations by numbers of live implants and early and late fetal deaths.	Study authors reported no significant evidence of dominant lethal mutations at any dose level.	These results suggest that BHT in not mutagenic to ICR/Ha Swiss mice.	Epstein et al. (1972)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	Male *Drosophila melanogaster* were exposed to 0.5% BHT and X-irradiated or treated with diepoxybutane or diethylnitrosamine to determine effects of BHT on frequency of sex-linked recessive lethal mutations, autosomal translocations, sex chromosome losses, or dominant lethals.	Study authors reported a lower frequency of sex-linked recessive lethals and translocations in *Drosophila* spermatids treated with X-rays and BHT. No significant effects of BHT on the induction of dominant lethals, sex-linked recessive lethals, or sex chromosome losses in mature spermatozoa treated with X-rays were reported. Spermatids treated with BHT and diepoxybutane or diethylnitrosamine demonstrated significantly fewer sex-linked recessive lethal mutations.	These results suggest inhibitory effects of BHT on mutagenic effects in spermatids of *Drosophila melanogaster*.	Sankaranarayanan (1983)
Genotoxicity	*B. subtilis* strains H-17 (rec^+) and M-45 (rec^-) were exposed to an unknown concentration of BHT in a rec assay. Differences in inhibition length were recorded and used to identify mutagenic effects.	Study authors reported no differences in inhibition length.	These results suggest no mutagenic effects of BHT on *B. subtilis*.	Kinae et al. (1981)
Genotoxicity	Salmonella strains TA98, TA100, and TA1537 were exposed to 10 µg/plate BHT in an Ames reversion assay. Cultures were evaluated for mutagenic effects with and without metabolic activation by S9 Wistar rat liver fraction.	Study authors reported negative results for all strains with and without metabolic activation.	These results suggest no mutagenic effects of BHT on Salmonella strains TA98, TA100, or TA1537.	Kinae et al. (1981)

Stokes and Scudder (1974) published a peer-reviewed, behavioral development dietary exposure study in which they exposed multigenerations of male and female Swiss Webster mice (age and weight not reported) to BHT (purity not stated). Pups from mated pairs were culled to eight pups per pair and weaned at 21 days of age, and then performed several behavioral tests. Mice were housed eight per cage at a constant temperature (21º C), with a 16-hour light/8-hour dark cycle. Water and food were available ad libitum. Mated pairs and pups were fed Purina Rat Chow and Pet Milk. Groups of 12 mice were randomly selected for a control group and an exposure group. The exposed parents received 0.5% (5000 ppm) BHT by diet weight. Pups were weaned at 21 days of age and exposed to 0.5% (5000 ppm) BHT by diet. Water and food were provided ad libitum. This study's conformance with GLP guidelines could not be determined. The dosing regimen is not explicit in the methods of the study in terms of the fetus' exposure time.

At 6 weeks of age, mice were selected randomly for behavioral testing (Stokes and Scudder, 1974). Social behavior was tested by placing naïve pairs of male and female mice into six small chambers, and interaction was recorded for 80 minutes. Study authors noted all instances of contractual behavior, digging, stereotypic behavior, freezing, ingestion, carrying, being groomed, grooming self, grooming others, sleeping, exploration, and aggression. Second, at 7 weeks of age, to test learning, mice were placed in an automated avoidance conditioning climbing screen where they were shocked every 5 seconds if they did not climb to a higher platform. Third, 10 males from each test group were kept isolated for 3 weeks and then tested for isolation-induced aggression. In conjunction with the third experiment, one group of 10 males, previously untreated, was fed the 0.5% (5000 ppm) BHT diet for the last 7 days of the 3-week study to evaluate acute effects on aggression. Study authors evaluated statistical significance for all tests, except for aggression results, using Student's *t*-test. The Wilcoxon two-sample rank test was used to determine statistical significance of the isolation-induced aggression testing.

Stokes and Scudder (1974) found that 0.5% BHT administered by diet significantly decreased sleeping and increased fighting compared to controls. Additionally, animals exposed to BHT did not exhibit learned behaviors in the automated avoidance conditioning climbing screening. Last, males showed significant increases in isolation-induced aggression when compared to controls. Table B.45 provides the mean and standard deviation of measured instances of behavior in the control and 0.5% exposure group. Study authors stated that due to animals being exposed pre- and postnatally to BHT it was not possible to determine which stage caused the adverse effects. The study authors believed that the change in aggression behavior was developmental because these effects were not seen in mice that were not treated in utero, but were administered 0.5% (5000 ppm) via diet for 7 days. The study authors further estimated that BHT may be largely lipophilic in nature and therefore interferes with the noted synaptic vesicle membrane.

In a study conducted by Holder et al. (1970), the biliary metabolism of BHT in the rat was examined and compared to the biliary metabolism of other BHT compounds—3,5-di-t-butyl-4-hydroxybenzyl alcohol (BHT-CH2OH); 3,5-di-t-butyl-4-hydroxybenzaldehyde (BHT-CHO); 3,5-di-t-butyl-4-hydroxybenzoiac acid (BHT-COOH); and 1,2-bis(3,5-di-t-butyl-4-hydroxypheny1)ethane (B-B). White male Wistar rats (total number of animals unclear) were given 100-µg doses of [^{14}C]-labeled BHT (or other amounts of its related compounds) in aqueous ethanol via intravenous (i.v.) or intraperitoneal (i.p.) injection. Animals were housed in

metabolism cages separated by glass (environmental conditions not specified) where urine and feces samples were collected. Bile was collected through a biliary cannula for periods of 6−8 hours. The study was conducted over a 5-day period.

Mean hourly biliary excretion values for radioactive BHT and its biliary metabolites (BHT-CH2OH, BHT-CHO, BHT-COOH, and B-B) were reported for 6 hours after a single intravenous dosing (six animals per compound, except for BHT-CHO, which included four animals) (Holder et al., 1970). Excretion values were recorded every 2 hours for an 8-hour period for the same compounds (except B-B) after single i.p. dosing (six animals for BHT and two animals for the other compounds). The results showed that these BHT compounds (with the exception of B-B) are rapidly absorbed, metabolized, and excreted in the bile.

Five days after single intraperitoneal dosing, hourly biliary excretion was measured over a 6-hour period for BHT-CH2OH, BHT-CHO, and BHT-COOH. After that 6-hour period (i.e., 126 hours after single i.p. dosing), the total recoveries (in % dose) for urine, feces, and bile of radioactive metabolites of BHT, BHT-CH2OH, BHT-CHO, and BHT-COOH were determined (numbers of animals varied) (Holder et al., 1970). There were individual differences among compounds in the ratio of urinary to fecal excretion of ^{14}C. However, there were no significant differences in the total radioactivity excreted over 126 hours after dosing. In general, approximately 70% of the dosed radioactivity for each BHT compound was recovered.

Potential differences in metabolism of i.v. administered BHT and i.p. administered BHT were suggested given differences in rates of excretion (Holder et al., 1970). Approximately 46% of i.v. administered BHT was excreted in the bile in the first hour, with 94% excreted 6 hours after dosing; approximately 32% of the administered i.p. dose was excreted in the bile 2 hours following dosing, with 52% excreted 6 hours after dosing. Furthermore, excretion data demonstrated that BHT-COOH and its ester glucuronide are the primary compounds in enterohepatic circulation.

Holder et al. (1970) identified the metabolites of BHT and its derivatives in urine, feces, and bile. In all biological extracts, BHT-COOH and/or ester glucuronide were the major metabolites present.

Stierum et al. (2008) examined the mechanism of BHT-induced liver changes in an in vivo assay using transcriptomic analysis. Groups of 7-week-old male Sprague-Dawley rats were treated with 0, 25, 75, 150, 300, or 1000 mg/kg-day BHT (purity 99.9%) via diet for 28 days (n = 10 for the control group and n = 6 for BHT-treated groups). The study authors reported that the actual dietary intakes based on animal body weight and food intakes in rats were 0, 28, 88, 167, 321, and 1159 mg/kg-day BHT. At study termination, livers of animals were dissected, and samples were obtained for cDNA microarray analysis. In addition, samples from the left lobe also were obtained for RT-PCR analysis. Sample selection for transcriptome analysis was made after histopathological and clinical chemistry analysis. For BHT, microarray analysis was performed using tissues obtained from the 150-, 300-, and 1000-mg/kg-day dose groups because no histological or clinical chemistry changes were observed at these doses. In addition to cDNA analysis, the study authors also determined CYP1A2 and CYP2B1/2 and glutathione *S*-transferase (GST) activity.

cDNA analysis indicated that the expression of 10 genes was impacted after treatment with BHT (Stierum et al., 2008). Hepatic mRNA levels of the phase 1 cytochrome P450 isozymes, CYP2B1/2, CYP3A9, and CYP2C6 were increased, with CYP2B1/2 levels exhibiting a clear dose-response trend. BHT also increased the mRNA levels of GST 1 among the phase 2 metabolizing enzymes in a dose-dependent manner. In addition to these phase 1 and 2 enzymes, mRNA levels of carboxylesterase 10 precursor, interleukin 15, hematopoietic cell tyrosine kinase, zinc finger protein 179, tryptophan-2-3-dioxygenase, and tropomyosin isoform 6 also were increased after BHT treatment. RT-PCR analysis indicated that there was a dose-dependent and statistically significant ($p < 0.001$) increase in mRNA levels of both CYP2B1 and CYP2B1/2 that correlated well with the cDNA microarray data. To confirm the cDNA microarray increase in GST μ2 levels after BHT treatment, the study authors conducted an assay for GST activity toward 1,2-dichloro-4-nitrobenzene (DCNB) as an indicator substrate. A dose-dependent and statistically significant ($p < 0.001$ at the three highest doses) increase was noted in GST μ2 activity toward DCNB compared to the concurrent control group. Based on these results, the study authors concluded that gene expression analysis provides new insights regarding the dose-dependent mode of action of BHT after in vivo administration in male rats. Induction of both phase 1 and 2 metabolizing enzymes may provide an understanding regarding the toxicity of BHT after oral administration.

In a published, peer-reviewed, comparative metabolism study (Matsuo et al., 1984), 5-week-old male and female DDY/Slc mice (4/sex/dose group) and 5-week-old male Sprague-Dawley rats (4/dose group) were given a single oral dose of BHT labeled at the *p*-methyl group with ^{14}C ([^{14}C]BHT; radiochemical purity >99%) of 20 or 500 mg/kg in a 5-mL corn oil suspension by stomach intubation. No information regarding GLP compliance was provided. The study authors also indicated that other dosing groups were examined. A group of male mice (number of animals unclear) was given daily oral doses of 20 mg [^{14}C]BHT/kg for 10 consecutive days for the purpose of tissue residue examination, and another group of 50 male mice was given a single oral dose of 500 mg [^{14}C]BHT/kg for the purpose of metabolite characterization.

For the duration of the experiment, animals were housed individually in metabolism cages in an air-conditioned room (25 ± 2°C) and supplied a diet of CE-2 of Clea Japan, Inc. and water, which were given ad libitum (Matsuo et al., 1984). Urine, feces, and expired air samples were collected. Seven days after treatment, the mice were sacrificed, and the following tissues were obtained: blood, heart, kidney, liver, spleen, pancreas, lung, brain, adrenal gland, muscle, sciatic nerve, spinal cord, salivary gland, fat, stomach, intestine, caecum, hair, skin, bone, testis, uterus, and ovary.

In mice given the single oral doses, absorption measured at 3 and 16 hours after treatment was highest in the stomach, intestines, gall bladder, and urinary bladder, and was present to a lesser extent in the liver, kidney, spleen, and salivary gland (Matsuo et al., 1984). At 24 hours minimal concentrations were found in the gall bladder, urinary bladder, liver, kidney, spleen, and digestive organs. By 168 hours, no concentrations were detected. Over the 7 days after treatment, 41−65% of [^{14}C]BHT was excreted in the feces, 26−50% in the urine, and 6−9% in expired air (total recovery 96−98%). For the 20 mg [^{14}C]BHT/kg single-dose group, ^{14}C-residue levels in tissues were low (<1 ppm). For the 500 mg [^{14}C]BHT/kg single-dose group, ^{14}C-residue levels in tissues were higher (up to 11 ppm). For mice given 20 mg [^{14}C]BHT/kg for 10 consecutive days, the study authors reported that tissue residues of ^{14}C increased over time

and appeared to move toward a steady state after 10 days. One to two days after treatment ended, a slight increment in tissue residues was found, after which tissue residues began to rapidly decrease. In male rats given a single oral dose of 20 or 500 mg, excretion of [^{14}C]BHT was 64−70% in feces and 16−19% in urine with the total recovery being 83−86% at 3 days following administration. Tissue residue analyses were not performed in rats.

More than 43 metabolites were identified in the urine and feces of both species (Matsuo et al., 1984). For mice and rats, the oxidation of the *p*-methyl group was the major metabolic reaction of [^{14}C]BHT. Oxidation of the *tert*-butyl groups was also a major metabolic reaction for mice, but this was only a minor reaction in rats.

Generally tests to assess the mutagenicity of BHT are negative. Ames assays testing the genotoxicity of BHT found no evidence of genotoxic effects in various strains of *S. typhimurium* (Bruce and Heddle, 1979; Shelef and Chin, 1980; Dertinger et al., 1993; Kinae et al., 1981) or *B. subtilis* (Kinae et al., 1981). McKee and Tometsko (1979) concluded that in nontoxic concentrations, BHT reduces chromosomal inversions by chemicals requiring metabolic activation. Examination of frequencies of sex-linked recessive lethal mutations in *Drosophila melanogaster* exposed to BHT in conjunction with radiation revealed radiosensitizing properties of BHT, but did not suggest BHT-induced genetic damage (Kamra, 1973; Barnett and Muñoz, 1980; Prasad and Kamra, 1974; Sankaranarayanan, 1983). Shamberger et al. (1973) and Paschin and Bahitova (1984) reported evidence of inhibitory effects of BHT on chromosomal breakage induced by exposure to dimethylbenzanthracene in human blood leukocytes and benzo(a)pyrene in hamster V79 cells, respectively. Epstein et al. (1972) found no evidence of mutagenic effects of BHT in mice. Sheu et al. (1988) also reported no mutagenic effects of BHT in mice, but observed an increase in dominant lethal mutations in rats exposed to BHT.

Based on the results of studies presented in Table 3, a mode of action for BHT cannot be determined.

DERIVATION OF PROVISIONAL VALUES

DERIVATION OF ORAL REFERENCE CONCENTRATIONS

Table 4 presents a summary of noncancer reference values. Table 5 presents a summary of cancer values. The cancer and inhalation toxicity values are converted to HEC/HED units, and the conversion process is presented as footnotes. IRIS data are indicated in the table if available.

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

The study by Olsen et al. (1986) is selected as the principal study for the derivation of the subchronic p-RfD. The study is published and peer-reviewed. Details are provided in the "Review of Potentially Relevant Data" section. The critical effect selected is decreased body weight observed at 9 weeks of treatment in F1 Wistar rats directly exposed to BHT via diet for 141−144 weeks (see Table B.22). This endpoint is supported by decreased body weight reported in several other studies of subchronic duration (Fulton et al., 1980, Powell et al., 1986; Hirose et al., 1993; NCI, 1979a,b; McFarlane et al., 1997a,b).

Table 4. Summary of Noncancer Reference Values for Butylated Hydroxytoluene (CASRN 128-37-0)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value[a]	POD Method	POD[b]	UF_C	Principal Study
Subchronic p-RfD (mg/kg-day)	Rat/M	Decreased F1 body weight at 9 weeks	1×10^{0}	NOAEL	100	100	Olsen et al. (1986)
Chronic p-RfD (mg/kg-day)	Rat/M	Decreased F1 adult weight at 138 weeks	3×10^{-1}	NOAEL	25	100	Olsen et al. (1986)
Subchronic p-RfC (mg/m^3)	None						
Chronic p-RfC (mg/m^3)	None						

[a]p-reference values are presented in mg/kg-day.
[b]Dosimetry: POD values are converted from a discontinuous to a continuous (daily) exposure in mg/kg-day.

Table 5. Summary of Cancer ReferenceValues for Butylated Hydroxytoluene (CASRN 128-37-0)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value[a]	Principal Study
p-OSF	Rat/M	Total hepatocellular tumors	3.6×10^{-3} $(mg/kg\text{-}day)^{-1}$	Olsen et al. (1986)
p-IUR	No information available			

[a]Dosimetry: POD values are converted from a discontinuous to a continuous (daily) exposure in mg/kg-dayand further converted to an HED.

Based on the available database (see Table 2), it appears that the liver is a target organ of BHT toxicity. Although there is support for a role of BHT in causing liver injury, liver effects may not be the most sensitive toxicological endpoint due to BHT exposure. The most sensitive subchronic liver endpoint appears to be increased relative liver weight in male Wistar rats with a LOAEL of 30 mg/kg-day from the Fulton et al. (1980) study. However, this LOAEL is not consistent with other values observed for increased relative liver weight from other experimental studies. In a chronic-duration study by Williams et al. (1990), a NOAEL of 79 mg/kg-day with a corresponding LOAEL of 237 mg/kg-day are identified for increased relative liver weight in male F344 rats. Second, a NOAEL of 147 mg/kg-day and a LOAEL of 368 mg/kg-day are identified for increased relative liver weight in male Wistar rats from the chronic-duration study by Deichmann et al. (1955d). The LOAELs from the Williams et al. (1990) and the Deichmann et al. (1955d) studies are nearly 7-fold higher than that identified for increased relative liver weight in male Wistar rats from the Fulton et al. (1980) study, suggesting that the LOAEL of 30 mg/kg-day may not be reliable because of its inconsistency with other values identified for the same endpoint. The next most sensitive subchronic value, a NOAEL of 250 mg/kg-day for increased incidences of nonneoplastic hepatic lesions (i.e., necrosis, fibrosis, hepatocyte hypertrophy, and hepatocyte hyperplasia) identified from the 28 day study by Powell et al. (1986), could be a potential POD for derivation of the subchronic p-RfD.

Another common toxicological effect of BHT is decreased body weight (see Table 2), which may be a more sensitive endpoint of BHT exposure than liver. From the developmental and reproductive study by McFarlane et al. (1997b), a LOAEL of 100 mg/kg-day is determined from this study by causing a 20% decrease in fetal body weight with a corresponding NOAEL of 25 mg/kg-day (see Table B.38). However, the effect of BHT on decreased fetal body weight is not consistent with other studies. Olsen et al. (1986) observed no BHT-related biologically significant effects on fetal body weight (see Table B.21). These data suggest that the decreased fetal body weight observed in the McFarlane et al. (1997b) study may not be a toxicological effect of BHT exposure and thus should not be used for derivation of a reference value.

Although Olsen et al. (1986) observed no changes in fetal body weight, decreased body weight was noted throughout the 141−144 weeks in which F1 rats were exposed to BHT via diet (see Table B.22). Because body-weight data were recorded throughout the study, subchronic effects of BHT can be delineated even though the study was performed with the purpose of determining chronic effects. At 9 weeks of exposure (i.e., subchronic-duration), there was a biologically (≥10% change) and statistically significant decrease in body weight in male and female rats at the highest dose tested (i.e., 250 mg/kg-day). These data from Olsen et al. (1986) are not amenable to BMD modeling because no data variability is provided, which is necessary for BMDS. A NOAEL of 100 mg/kg-day is identified from this study for an 11% decrease in body weight in male rats with a corresponding LOAEL of 250 mg/kg-day. The selection of this NOAEL of 100 mg/kg-day as the POD would protect against BHT-decreased body weight but also the liver effects observed in the 28 day study by Powell et al. (1986). Therefore, the critical effect selected is decreased body weight at 9 weeks in F1 male Wistar rats (Olsen et al., 1986). The NOAEL of 100 mg/kg-day based on this effect is chosen as the POD to derive a subchronic p-RfD.

Adjusted points for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for dietary administration. Dosimetric adjustment for 100 mg/kg-day is presented below.

$$\begin{aligned}(DOSE_{ADJ}) &= DOSE_{\text{Olsen et al. 1986}} \times [\text{conversion to daily dose}] \\ &= 100 \text{ mg/kg-day} \times (\text{days of week dosed} \div 7) \\ &= 100 \text{ mg/kg-day} \times (7 \div 7) \\ &= 100 \text{ mg/kg-day}\end{aligned}$$

A subchronic p-RfD for BHT, based on a NOAEL of 100 mg/kg-day in male rats (Olsen et al., 1986), is developed as follows:

$$\begin{aligned}\textbf{Subchronic p-RfD} &= \textbf{NOAEL} \div \textbf{UF}_\textbf{C} \\ &= 100 \text{ mg/kg-day} \div 100 \\ &= \mathbf{1 \times 10^0 \text{ mg/kg-day}}\end{aligned}$$

Tables 6 and 7, respectively, summarize the uncertainty factors and the confidence descriptor for the subchronic p-RfD for BHT.

Table 6. Uncertainty Factors for Subchronic p-RfD of Butylated Hydroxytoluene

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to subchronic oral exposure to BHT.
UF_D	1	A UF_D of 1 is selected because the database includes one acceptable three-generation reproduction study in mice (Tanaka et al., 1993) and two developmental studies in rats and mice (McFarlane et al., 1997).
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied for using a POD based on a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic study was utilized as the principal study.
UF_C	100	

[a]Olsen et al. (1986).

Table 7. Confidence Descriptor for Subchronic p-RfD for Butylated Hydroxytoluene

Confidence Categories	Designation[a]	Discussion
Confidence in the study	H	Confidence in the key study is high. Olsen et al. (1986) is a toxicity study in rats that was performed to investigate developmental, reproductive, and carcinogenic effects of BHT administration on Wistar rats. Multiple studies in the literature support decreased body weight as an index of BHT toxicity.
Confidence in the database	H	Confidence in the database is high. The database includes subchronic and chronic toxicity studies in four species (rat, mouse, hamster, and dog), developmental toxicity studies in two species (rat and mouse), and a two-generation reproduction study (mouse).
Confidence in the subchronic p-RfD[b]	H	The overall confidence in the subchronic p-RfD is high.

[a]L = low; M = medium; H = high.
[b]The overall confidence cannot be greater than the lowest entry in the table.

The confidence of the subchronic p-RfD for BHT is high, as explained in Table 7.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

To fully evaluate noncancer effects due to chronic exposure of BHT, BMD modeling was performed on appropriate data from chronic studies listed in Table 2. The studies for each effect are presented in Table 8 with their corresponding benchmark responses (BMRs), BMDs, and BMDLs.

Whenever possible, dose-response models were fit to the data from the aforementioned studies to estimate potential PODs from which to derive the RfD. All of the common models (i.e., Gamma, Multistage, Logistic, Probit, Weibull, and Quantal-Linear models for dichotomous data; Linear, Polynomial, Power, and Hill models for continuous data) available in the EPA's Benchmark Dose Software (BMDS, version 2.1.2) were fit to the data, and results are summarized in Table 8 below. A detailed summary of BMD methodology and modeling results is provided in Appendix C. These results and their associated studies were reviewed to establish a principal study and a POD.

The study by Olsen et al. (1986) is selected as the principal study for the derivation of the chronic p-RfD. The study is published and peer-reviewed. Details are provided in the "Review of Potentially Relevant Data" section. The critical effect selected is decreased body weight at 138 weeks in F1 Wistar rats exposed to BHT via diet for 141−144 weeks (see Table B.22). This study provides the most sensitive toxicological endpoint.

BMD modeling was performed as described in Appendix C, to fully evaluate noncancer effects due to chronic exposure of BHT. After reviewing the modeling results, it was determined that liver peliosis in male mice from the NCI (1979d) study, was the most sensitive liver effect due to chronic exposure of BHT. For the endpoint of increased incidence of liver peliosis, the Log-logistic model was considered the best fit and produced a BMD_{10} and $BMDL_{10}$ of 73 and 14 mg/kg-day (see Table 8). However, the $BMDL_{10}$ of 14 mg/kg-day is 37-fold lower than lowest dose (i.e., 515 mg/kg-day) tested for this effect, suggesting that the BMD modeling of

liver peliosis is not reliable and the resulting $BMDL_{10}$ is not suitable as a potential POD. A similar situation was determined for the liver necrosis data with a $BMDL_{10}$ of 16 mg/kg-day. The next most sensitive and reliable, possible POD for liver effects from the NCI (1979d) study is a $BMDL_{10}$ of 100 mg/kg-day for increased incidence of hepatic cytoplasmic vacuolation in male rats.

In the study by Olsen et al. (1986), biologically and statistically decreases in body weight were observed in F1 Wistar rats, throughout the duration of the experiment. At the end of 138 weeks, a NOAEL of 25 mg/kg-day is identified with a corresponding LOAEL of 100 mg/kg-day for an 11% decrease in body weight of male rats. Therefore, the critical effect selected is decreased body weight at 138 weeks in F1 male Wistar rats (Olsen et al., 1986). The NOAEL of 25 mg/kg-day based on this effect is chosen as the POD to derive a chronic p-RfD. The selection of the NOAEL of 25 mg/kg-day based on chronic body-weight depression as the POD will not only protect against this effect but also the liver effects of BHT observed in the NCI (1979d) study.

Table 8. BMDLs for Multiple Noncancer Effects Following Chronic Oral Exposure to BHT

	Dose (mg/kg-day)			
	BMR[a]	BMD	BMDL	Comments
NCI (1979c) Focal alveolar histiocytosis	10%	199 (F)	157 (F)	Increased incidence of focal alveolar histiocytosis in female F344 rats. M and F notations in this case refer to the sex of rats.
NCI (1979d) Hepatocytomegaly	10%	355 (M)	284 (M)	Increased incidence of hepatocytomegaly in $B6C3F_1$ mice. M and F notations in this case refer to the sex of mice.
NCI (1979d) Liver peliosis	10%	73 (M)	14 (M)	Increased incidence of liver peliosis in $B6C3F_1$ mice. M and F notations in this case refer to the sex of mice. BMD results are not reliable.
NCI (1979d) Liver necrosis	10%	142 (M)	16 (M)	Increased incidence of liver necrosis in $B6C3F_1$ mice. M and F notations in this case refer to the sex of mice. BMD results are not reliable.
NCI (1979d) Hepatic cytoplasmic vacuolation	10%	180 (M)	100 (M)	Increased incidence of hepatic cytoplasmic vacuolation in $B6C3F_1$ mice. M and F notations in this case refer to the sex of mice.
McFarlane et al. (1997a) Body weight of F0 dams	10%	730	664	Decreased body weight in F0 Wistar rat dams.
McFarlane et al. (1997a) Relative liver weight	10%	No fit	No fit	Increased relative liver weight in Wistar rat dams.
McFarlane et al. (1997a) Body weight of pups	10%	No fit	No fit	Decreased body weight in Wistar pups.

[a]BMR = benchmark response.

BMD input data for these liver data are presented in Tables B.18, B.30, B.34, and B.35. The curves and BMD output text are provided in Appendix C.

Adjusted points for daily exposure:

Daily doses in mg/kg food were provided by Olsen et al. (1986). The following dosimetric adjustments were made for each dose in the principal study for dietary administration. Dosimetric adjustment for 25 mg/kg-day is presented below.

$$\begin{aligned}(DOSE_{ADJ}) &= DOSE_{\text{Olsen et al. 1986}} \times [\text{conversion to daily dose}] \\ &= 25 \text{ mg/kg-day} \times (\text{days of week dosed} \div 7) \\ &= 25 \text{ mg/kg-day} \times (7 \div 7) \\ &= 25 \text{ mg/kg-day}\end{aligned}$$

The chronic p-RfD for BHT, based on the NOAEL of 25 mg/kg-day in male mice (Olsen et al, 1986), is derived as follows:

$$\begin{aligned}\textbf{Chronic p-RfD} &= \textbf{NOAEL} \div \textbf{UF}_C \\ &= 25 \text{ mg/kg-day} \div 100 \\ &= \mathbf{3 \times 10^{-1} \text{ mg/kg-day}}\end{aligned}$$

Tables 9 and 10, respectively, summarize the uncertainty factors and the confidence descriptor for the chronic p-RfD for BHT.

Table 9. Uncertainty Factors for Chronic p-RfD of Butylated Hydroxytoluene (Olsen et al., 1986)

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between mice and humans. There are no data to determine whether humans are more or less sensitive than mice to chronic oral exposure to BHT.
UF_D	1	A UF_D of 1 is selected because the database includes one acceptable three-generation reproduction study in mice (Tanaka et al., 1993) and two developmental studies in rats and mice (McFarlane et al., 1997).
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	1	A UF_S of 1 is applied because a chronic study was utilized as the principal study.
UF_C	100	

The confidence of the chronic p-RfD for BHT is high, as explained in Table 10.

Table 10. Confidence Descriptor for Chronic p-RfD for Butylated Hydroxytoluene

Confidence Categories	Designation[a]	Discussion
Confidence in the study	H	Confidence in the key study is high. Olsen et al. (1986) is a toxicity study in rats that was performed to investigate developmental, reproductive, and carcinogenic effects of BHT administration on Wistar rats. Multiple studies in the literature support decreased body weight as an index of BHT toxicity.
Confidence in the database	H	Confidence in the database is high. The database includes subchronic and chronic toxicity studies in four species (rat, mouse, hamster, and dog), developmental toxicity studies in two species (rat and mouse), and a two-generation reproduction study (mouse).
Confidence in the chronic p-RfD[b]	H	The overall confidence in the chronic p-RfD is high.

[a]L = low; M = medium; H = high.
[b]The overall confidence cannot be greater than the lowest entry in the table.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No human or animal studies examining the toxicity of BHT following inhalation exposure have been identified. Therefore derivation of a subchronic p-RfC or chronic p-RfC is precluded.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Cancer Weight-of-Evidence Descriptor

Table 11 identifies the cancer weight-of-evidence descriptor for BHT.

Table 11. Cancer Weight-of-Evidence Descriptor for Butylated Hydroxytoluene[a]

Possible WOE Descriptor	Designation[b]	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	N/A	N/A	No human cancer studies are available.
"Likely to be Carcinogenic to Humans"	N/A	N/A	The evidence from animal studies is mixed with both positive and negative results regarding the carcinogenic potential of BHT. Therefore, BHT cannot be considered "*Likely to be carcinogenic to humans*."
"Suggestive Evidence of Carcinogenic Potential"	**Selected**	**Oral**	**Liver tumors related to BHT treatment have been reported in both male and female rats (Olsen et al., 1986) as well as in male mice (Inai et al., 1988). However, there are also four animal studies that reported negative tumor findings in rodents treated with BHT (Williams et al. 1990; NCI, 1979c; Price, 1994). Therefore based on the mixed results from the available cancer studies, BHT is considered to have "*Suggestive evidence of carcinogenic potential.*"**
"Inadequate Information to Assess Carcinogenic Potential"	N/A	N/A	Adequate information is available to assess carcinogenic potential.
"Not likely to be Carcinogenic to Humans"	N/A	N/A	No evidence of noncarcinogenicity is available.

[a]Bold text indicates choice of cancer weight-of-evidence descriptor.
[b]N/A = not applicable.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

As noted in Table 11, EPA concluded that there is *suggestive evidence of carcinogenic potential* for BHT. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) state: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates."

In the case of BHT, although there are no epidemiologic studies that have evaluated the carcinogenicity in humans, the carcinogenicity of BHT has been evaluated in several studies in both rats and mice. As described in Table 11, these studies indicate that there are mixed results regarding the carcinogenic potential of BHT. However, the study by Olsen et al. (1986) is a well-conducted study showing evidence of increased incidence of tumors in both sexes of one species at multiple exposure levels, and the data from this study are adequate to support a quantitative cancer dose-response assessment. Considering these data and uncertainty associated

with the suggestive nature of the tumorigenic response, it was concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of BHT is deemed appropriate.

The rat study by Olsen et al. (1986) is selected as the principal study for derivation of the p-OSF. The critical endpoint is increased incidence of total hepatocellular tumors (sum of adenomas and carcinomas) in F1 Wistar rats. This study is well conducted, peer-reviewed, and data from this study can support a quantitative cancer dose-response assessment. It is, however, unclear if this study was performed according to GLP standards. Details are provided in the "Selection of Potentially Relevant Studies" section.

Olsen et al. (1986) conducted a two-generation feeding study that yielded evidence of BHT-induced cancer. Dose-related increases of hepatocellular adenomas, carcinomas, and total hepatocellular tumors were reported in male and female (only increases in adenomas and total tumors) rats that had been exposed in utero, during lactation, and through 141−144 weeks of life. The data for increased incidence of hepatocellular carcinomas in male rats is not amenable to BMD modeling because there are no data at the low response range which is necessary for BMD modeling. Therefore, only the dose-response data for hepatocellular adenomas and total hepatocellular tumors (sum of adenomas and carcinomas) in male and female rats can be used to derive a p-OSF for BHT (see Tables 13 and B.25). The curves and BMD output text are provided in Appendix D. Table 14 presents a summary of the results for the BMD modeling. The incidence of total hepatocellular tumors in male rats was considered the most sensitive tumor response because the modeled data produced a slightly lower BMD_{10} and $BMDL_{10}$ of 41 and 28 mg/kg-day, respectively, compared to other tumor data (i.e., total tumors and adenomas) from male and female rats. The selection of total hepatocellular tumors as the critical endpoint is supported by the results of Inai et al. (1988), who reported increased liver tumors in male mice, as well as other pathologies noted in the liver (liver enlargement, increased xenobiotic liver enzymes, vacuolation of hepatocytes, liver necrosis, and peliosis), which may indicate a nongenotoxic mode of action for tumor formation (OECD SIDS, 2002). However, there are currently insufficient data to support a carcinogenic mode of action for BHT.

Table 12 summarizes the relevant oral carcinogenicity studies for BHT.

Table 12. Summary of Positive Oral Cancer Studies For Butylated Hydroxytoluene

References	# M/F, Species	Exposure (mg/kg-day)	Frequency/ Duration	$NOAEL_{HED}$[a] (mg/kg-day)	$LOAEL_{HED}$[a] (mg/kg-day)	Critical Endpoint
Olsen et al. (1986)	60/60 F1 Wistar rat	F1 Males: 0, 7.1, 28, 69 F1 Females: 0, 6.4, 25, 62	7 d/wk for 141−144 weeks in diet	N/A	N/A	Increased incidence of hepatocellular tumors (hepatocellular adenomas and carcinomas in males and adenomas in females)
Inai et al. (1988)	50/50 $B6C3F_1$ mouse	Males: 0, 249, 529 Females: 0, 262, 619	7 d/wk for 104 weeks in diet	N/A	N/A	Increased incidence of liver adenomas in males

[a]Dosimetry: NOAEL and LOAEL values are converted to human equivalent dose (HED in mg/kg-day). All exposure values are converted from a discontinuous to a continuous (weekly) exposure. Values for oral (cancer only) are further converted to an HED using the following equation: HED = Dose × (Body Weight Animal ÷ Body Weight Human)$^{1/4}$.

N/A= not applicable.

Adjusted points for daily exposure:

Daily doses in mg/kg-day were provided by Olsen et al. (1986). The following dosimetric adjustments were made for dietary treatment in adjusting doses for oral cancer analysis (see example calculation below):

$DOSE_{HED}$	**=**	**Dose × (Body Weight Animal ÷ Body Weight Human)$^{0.25}$**
Body-weight adjustment	=	$(BW_A \div BW_H)^{0.25}$
BW_H	=	70 kg (human reference body weight (U.S. EPA, 1997)
BW_A	=	0.468 kg (time-weighted average body weight for male Wistar rats calculated using body-weight data from Olsen et al., 1986)
Body-weight adjustment	=	$(0.468 \div 70)^{0.25} = 0.285$
$DOSE_{HED}$	=	$(Dose)_n \times 0.285$
	=	25 mg/kg-day × 0.285
	=	**7.1 mg/kg-day**

Table 13 presents the BMD input for incidence of total hepatocellular tumors (sum of adenomas and carcinomas) in male and female rats exposed to BHT for 141−144 weeks.

Table 13. Dose-Response Data for Butylated Hydroxytoluene-Induced Total Hepatocellular Tumors (Adenomas and Carcinomas) in Rats Exposed via Diet for 141−144 Weeks[a]

Dose (mg/kg-day)	$Dose_{HED}$ (mg/kg-day)[b]	Incidence
Male Rats		
0	0	2/100
25	7.1	1/80
100	28	6/80
250	69	26/99
Female rats		
0	0	2/100
25	6.4	3/79
100	25	6/80
250	62	14/99

[a]Olsen et al. (1986).
[b]Doses converted to human equivalency doses using:
$HED = Dose \times (Body\ Weight\ Animal \div Body\ Weight\ Human)^{1/4}$.

Table 14. Goodness-of-Fit Statistics, BMD_{10HED}, and $BMDL_{10HED}$ Values for a Dichotomous Model for Hepatocellular Tumors in F1 Wistar Rats Treated with BHT in the Diet for 141−144 weeks[a,b]

Model	Goodness-of-Fit *p*-Value[c]	AIC for Fitted Model	BMD_{10HED} (mg/kg-day)	$BMDL_{10HED}$ (mg/kg-day)	Conclusions
Multistage-Cancer Male (adenomas)	0.667	159.432	42	30	
Multistage-Cancer Female (adenomas)	0.906	165.063	58	36	
Multistage-Cancer Male (total tumors)	**0.496**	**193.48**	**41**	**28**	**Lowest BMDL**
Multistage-Cancer Female (total tumors)	0.979	172.471	49	32	

[a]Olsen et al. (1986).
[b]Bold text indicates BMD model selected to derive the p-OSF.
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria.

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose.

p-OSF = **0.1 ÷ $BMDL_{10}$**
= 0.1 ÷ 28 mg/kg-day
= **3.6×10^{-3} $(mg/kg\text{-}day)^{-1}$**

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies examining the carcinogenicity of BHT following inhalation exposure have been identified. Therefore, derivation of a p-IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

No screening values are presented.

APPENDIX B. DATA TABLES

Table B.1. Concentration of BHT in Tissues of Male Wistar Rats Administered Butylated Hydroxytoluene by Gavage for 7 or 28 Days

Parameter	Exposure Group			
	0 mg/kg-day	**25 mg/kg-day**	**250 mg/kg-day**	**500 mg/kg-day[a]**
7-day study				
Liver (mg/kg wet weight)	<1	<1	<1	<1[b]
Epididymal adipose tissue (mg/kg wet weight)[c]	<0.5	11.0 ± 0.70	66.6 ± 25.24	227.4 ± 97.70
28-day study				
Liver (mg/kg wet weight)	<1	<1	<1	<1
Epididymal adipose tissue (mg/kg wet weight)[c]	<0.5	15.5 ± 3.22	119.8 ± 28.93	168.4 ± 36.51

[a]The 500-mg/kg-day dose group was treated with 750 mg/kg-day for the first three days of the exposure period, resulting in an average daily dose of 607 mg/kg-day for the 7-day study and 527 mg/kg-day for the 28-day study. The equation used for calculating the daily average is:
$Dose_{ADD}$ = [(daily dose (mg/kg-day) × days dosed) + (daily dose (mg/kg-day) × days dosed)] ÷ total days.
[b]Except in two rats, with 8.3 and 17.9 mg BHT/kg wet liver, respectively.
[c]Mean ± SD.

Source: Powell et al. (1986).
Notes: Quantitative statistics not provided for this data from the study and cannot be performed independently due to lack of information.

Table B.2. Liver Biochemistry in Male Wistar Rats Administered Butylated Hydroxytoluene by Gavage for 7 or 28 Days

Parameter	Exposure Group			
	0 mg/kg-day	**25 mg/kg-day**	**250 mg/kg-day**	**500 mg/kg-day[a]**
7-day study				
Hepatic microsomal protein yield (mg/g wet weight liver)[b]	20.0 ± 2.31	17.7 ± 2.88 (89)	19.0 ± 1.81 (95)	15.3 ± 2.63 (77)[c]
Hepatic glucose 6-phosphatase activity (µmol/min/mg whole homogenate protein)[b]	0.05 ± 0.007	0.06 ± 0.003 (120)	0.05 ± 0.003 (100)	0.03 ± 0.009 (60)[c]
Hepatic cytochrome b_5 concentration (nmol/mg microsomal protein)[b]	0.52 ± 0.09	0.61 ± 0.06 (117)	0.65 ± 0.06 (125)[c]	0.59 ± 0.10 (113)
28-day study				
Hepatic microsomal protein yield (mg/g wet weight liver)[b]	24.0 ± 3.16	23.7 ± 3.44 (99)	28.2 ± 3.24 (118)[c]	29.5 ± 1.50 (123)[c]
Hepatic glucose 6-phosphatase activity (µmol/min/mg whole homogenate protein)[b]	0.04 ± 0.002	0.04 ± 0.003 (100)	0.03 ± 0.003 (75)[c]	0.03 ± 0.002 (75)[c]
Hepatic cytochrome b_5 concentration (nmol/mg microsomal protein)[b]	0.55 ± 0.04	0.62 ± 0.08 (113)[c]	0.66 ± 0.06 (120)[c]	0.57 ± 0.05 (104)

[a]The 500-mg/kg-day dose group was treated with 750 mg/kg-day for the first three days of the exposure period, resulting in an average daily dose of 607 mg/kg-day for the 7-day study and 527 mg/kg-day for the 28-day study. The equation used for calculating the daily average is:
$Dose_{ADD}$ = [(daily dose (mg/kg-day) × days dosed) + (daily dose (mg/kg-day) × days dosed)] ÷ total days.
[b]Mean ± SD, (corresponding percentage of control); calculated for this review.
[c]Significantly different from control ($p < 0.05$) by the Student's *t*-test conducted by the study authors.

Source: Powell et al. (1986).

Table B.3. Hepatic Lesions in Male Wistar Rats Administered Butylated Hydroxytoluene by Gavage for 7 or 28 Days

Parameter		Exposure Group		
		25 mg/kg-day	250 mg/kg-day	500 mg/kg-day[a]
7-day study				
Periportal region[b]	Hepatocyte necrosis[c]	0/5 (0)	0/5 (0)	2/5 (40)
	Fibrosis[c]	0/5 (0)	0/5 (0)	3/5 (60)
	Hepatocyte hypertrophy[c]	0/5 (0)	0/5 (0)	3/5 (60)
	Hepatocyte hyperplasia[c]	0/5 (0)	0/5 (0)	4/5 (80)
	Glycogen accumulation[c]	0/5 (0)	4/5 (80)	4/5 (80)
28-day study				
Periportal region[b]	Hepatocyte necrosis[c]	0/10 (0)	0/10 (0)	6 /10 (60)
	Fibrosis[c]	0/10 (0)	0/10 (0)	5/10 (50)
	Bile-duct cell proliferation[c]	0/10 (0)	0/10 (0)	4/10 (40)
	Hepatocyte hypertrophy[c]	0/10 (0)	0/10 (0)	2/10 (20)
	Hepatocyte hyperplasia[c]	0/10 (0)	0/10 (0)	3/10 (30)
	Pigment-laden macrophages[c]	0/10 (0)	0/10 (0)	3/10 (30)
	Glycogen depletion[c]	0/10 (0)	0/10 (0)	7/10 (70)
	Glycogen accumulation[c]	0/10 (0)	8/10 (80)	0/10 (0)
Mid-zonal glycogen accumulation[c]		0/10 (0)	0/10 (0)	5/10 (50)

[a]The 500-mg/kg-day dose group was treated with 750 mg/kg-day for the first three days of the exposure period, resulting in an average daily dose of 607 mg/kg-day for the 7-day study and 527 mg/kg-day for the 28-day study. The equation used for calculating the daily average is:
$Dose_{ADD}$ = [(daily dose (mg/kg-day) × days dosed) + (daily dose (mg/kg-day) × days dosed)] ÷ total days.
[b]No tests for significance were performed due to lack of control data.
[c]Number of animals with lesions/number examined per dose group, () –corresponding percentages; calculated for this review.

Source: Powell et al. (1986).

Table B.4. Mean Weight Gain and Relative Liver Weight of Male Wistar Rats Orally Exposed to Butylated Hydroxytoluene for 8 Weeks

Parameter	Exposure Group (Daily Dose, mg/kg-day)				
	0	0.02% (30)	0.10% (151)	0.50% (755)	0.75% (1132)
Initial body weight (g)[a]	37.0	39.2	43.4	48.1	51.6
Total food intake (g)[a]	472	509[b]	553[b]	435	461
Mean body-weight gain (g)[a]	159.6	164.4 (103)	152.6 (96)	77.8 (49)[b]	51.8 (32)[b]
Mean liver weight (g)[a]	6.3	7.7	8.2	6.7	5.4
Liver to body-weight ratio[a]	0.03	0.04 (↑33)	0.04 (↑33)	0.06 (↑100)	0.05 (↑67)

[a]Mean, (percent difference from control); calculated for this review.
[b]Significantly different from control ($p \leq 0.05$) by a partial correlation for multivariant data test conducted by the study authors.

Source: Fulton et al. (1980).

Table B.5. Ileal Biopsy Data of Male Wistar Rats Orally Exposed to Butylated Hydroxytoluene for 8 Weeks

Parameter		Exposure Group (Daily Dose, mg/kg-day)				
		0	**0.02% (30)**	**0.10% (151)**	**0.50% (755)**	**0.75% (1132)**
Villus:	Height (µm)[a]	230.6	221.9 (96)	179.2 (78)	147.8 (64)	137.3 (60)
	Goblet count[a]	22.3	23.0 (103)	14.2 (64)	13.0 (58)	13.3 (60)
Crypt:	Depth (µm)[a]	59.8	47.2 (79)	35.8 (60)	40.9 (68)	40.0 (67)
	Goblet count[a]	7.2	7.6 (106)	2.8 (39)	2.1 (29)	2.7 (38)

[a]Mean, (percentage of control); calculated for this review.

Source: Fulton et al. (1980)
Notes: Quantitative statistics were not provided for this data from the study and cannot be performed independently due to lack of information.

Table B.6. Survival and Weight Loss of Male and Female Fischer F344 Rats Following Neat Administration of Butylated Hydroxytoluene for 7 Weeks

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]				
	0 ppm	**6200 ppm (620)**	**12,500 ppm (1250)**	**25,000 ppm (2500)**	**50,000 ppm (5000)**
Male Rats					
Sample Size	5	5	5	5	5
Survival	5	5	4	5	0
Mean Weight (% of control)[b]	100	88	74	38	-
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]**				
	0 ppm	**6200 ppm (700)**	**12,500 ppm (1411)**	**25,000 ppm (2822)**	**50,000 ppm (5645)**
Female Rats					
Sample Size	5	5	5	5	5
Survival	5	5	5	5	0
Mean Weight (% of control)[b]	100	93	84	44	-

[a]Doses were converted to adjusted daily doses using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]At Week 7 as percentage of control.

Source: NCI (1979a).
Notes: Quantitative statistics were not provided for this data from the study and cannot be performed independently due to lack of information.

Table B.7. Survival and Weight Loss of Male and Female B6C3F$_1$ Mice After Neat Administration of Butylated Hydroxytoluene for 7 Weeks

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]					
	0 ppm	**3100 ppm (559)**	**6200 ppm (1118)**	**12,500 ppm (2255)**	**25,000 ppm (4509)**	**50,000 ppm (9019)**
Male Mice						
Sample Size	5	5	5	5	5	5
Survival	5	5	5	5	5	4
Mean Weight (% of control)[b]	100	89	94	78	79	73
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]**					
	0 ppm	**3100 ppm (605)**	**6200 ppm (1210)**	**12,500 ppm (2439)**	**25,000 ppm (4878)**	**50,000 ppm (9756)**
Female Mice						
Sample Size	5	5	5	5	5	5
Survival	5	5	5	5	4	1
Mean Weight (% of control)[b]	100	88	83	82	74	97

[a]Doses were converted to adjusted daily doses using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]At Week 7 as percentage of control.

Source: NCI (1979b).
Notes: Quantitative statistics were not provided for this data from the study and cannot be performed independently due to lack of information.

Table B.8. Body and Liver Weights of Fisher 344 Rats Administered Butylated Hydroxytoluene by Diet for 76 and 110 Weeks

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]					
	0 ppm (0)	100 ppm (8)	300 ppm (24)	1000 ppm (79)	3000 ppm (237)	6000 ppm (474)
76-week study						
Body weight (g)[b]	409 ± 35	424 ± 42 (104)	409 ± 34 (100)	411 ± 20 (100)	362 ± 35[c] (89)	367 ± 49[d] (90)
Liver weight (g)[b]	14.5 ± 2.1	14.2 ± 1.3 (98)	15.0 ± 1.0 (103)	15.2 ± 1.3 (105)	14. 7 ± 3.4 (101)	19.1 ± 3.3[e] (132)
Relative liver weight (g/100 g body weight)[b]	3.6 ± 0.5	3.4 ± 0.4 (94)	3.7 ± 0.3 (103)	3.7 ± 0.4 (103)	4.0 ± 0.6 (111)	5.4 ± 1.4[f] (150)

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]	
	0 ppm (0)	12000 (947)
110-week study		
Body weight (g)[b]	425 ± 35	379 ± 28[g] (89)
Liver weight (g)[b]	18.9 ± 3.8	15.6 ± 2.4[f] (83)
Relative liver weight (g/100 g body weight)[b]	4.4 ± 0.8	4.0 ± 0.5 (91)

[a]Doses were converted from then ppm intake in food is adjusted using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Mean ± SD, (percentage of control); calculated for this review.
[c]Significantly different from control ($p \leq 0.02$) by Student's *t*-test performed by the study authors.
[d]Significantly different from control ($p \leq 0.05$) by Student's *t*-test performed by the study authors.
[e]Significantly different from control ($p \leq 0.002$) by Student's *t*-test performed by the study authors.
[f]Significantly different from control ($p \leq 0.01$) by Student's *t*-test performed by the study authors.
[g]Significantly different from control ($p \leq 0.001$) by Student's *t*-test performed by the study authors.

Source: Williams et al. (1990).

Table B.9. Liver Lesions in Fisher 344 Rats Administered Butylated Hydroxytoluene by Diet for 76 and 110 Weeks

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]					
	0 ppm (0)	100 ppm (2)	300 ppm (6)	1000 ppm (21)	3000 ppm (64)	6000 ppm (129)
76-week study						
Hepatocellular Altered Foci						
Incidence (%)	1/4 (25)	1/4 (25)	1/4 (25)	1/4 (25)	1/4 (25)	1/4 (25)
Profiles[c] (No./cm^2)	0.1 ± 0.6	0.3 ± 0.6	0.3 ± 0.5	0.3 ± 0.6	0.4 ± 0.6	0.7 ± 1.2
Area[c] (mm^2)	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.5
Area (%)	0	0	0.03 ± 0.04	0	0.03 ± 0.04	0.03 ± 0.04
Neoplasms						
No. of adenomas	3	1	2	2	1	2
No. of carcinomas	0	0	0	0	0	0
Multiplicity[c]	0.5 ± 1.4	0.1 ± 0.3	0.3 ± 0.5	0.3 ± 0.5	0.1 ± 0.5	0.3 ± 0.5
Incidence (%)	3/7 (17)	1/7 (14)	2/7 (29)	2/7 (29)	1/7 (14)	2/6 (33)

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]	
	0 ppm (0)	12000 (257)
110-week study		
Hepatocellular Altered Foci		
Incidence (%)	16/25 (64)	9/23 (39)
Profiles[c] (No./cm^2)	0.9 ± 0.9	0.5 ± 0.8
Area[c] (mm^2)	0.2 ± 0.2	0.1 ± 0.1
Area[c] (%)	0.3 ± 0.3	0.1 ± 0.1
Neoplasms		
No. of adenomas	9	3
No. of carcinomas	0	0
Multiplicity[c]	1.0 ± 1.6	0.1 ± 0.3[d]
Incidence (%)	9/25 (36)	3/23 (13)

[a]Doses were converted from then ppm intake in food is adjusted using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Doses were converted from adjusted daily doses to human equivalency doses using the following formula:
$Dose_{HED}$ = $Dose_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)^(0.25).
[c]Mean ± SD.
[d]Significantly different from control ($p \leq 0.02$) using Fisher's exact probability test, as reported by the study authors.

Source: Williams et al. (1990).

Table B.10. Gastric Lesions in Fisher 344 Rats Administered Butylated Hydroxytoluene by Diet for 76 and 110 Weeks

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]			
	0 ppm (0)[c]	6000 ppm (129)[c]	0 ppm (0)[d]	12000 ppm (257)[d]
Squamous stomach hyperplasia				
Mild[e]	2/5 (40)	4/10 (40)	12/25 (48)	14/23 (61)
Moderate	0/5 (0)	0/10 (0)	0/25 (0)	0/23 (0)
Severe	0/5 (0)	0/10 (0)	0/25 (0)	0/23 (0)
Squamous stomach neoplasms				
Squamous cell papilloma	0/5 (0)	0/10 (0)	0/10 (0)	0/10 (0)
Gladular stomach dysplasia				
Mild	0/5 (0)	0/10 (0)	0/10 (0)	0/23 (0)
Moderate	0/5 (0)	0/10 (0)	0/10 (0)	0/23 (0)
Severe	0/5 (0)	0/10 (0)	0/10 (0)	0/23 (0)

[a]Doses were converted from then ppm intake in food is adjusted using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Doses were converted from adjusted daily doses to human equivalency doses using the following formula:
$Dose_{HED}$ = $Dose_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)^(0.25).
[c]Study period was 76 weeks.
[d]Study period was 110 weeks.
[e]Incidence/total number of animals, () –corresponding percentage; calculated for this review.

Source: Williams et al. (1990)

Table B.11. Final Body Weight, Organ Weight, and Food Consumption in F344 Male Rats Administered Butylated Hydroxytoluene by Diet for 36 Weeks

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]	
	0% (0)	0.7% (700)
Sample size	11	12
Body weight (g)[b]	402 ± 21	348 ± 16[c] (87)
Relative liver weight (g/100 g bw)[b]	2.69 ± 0.16	4.73 ± 0.22[c] (176)
Relative kidney weight (g/100 g bw)[b]	0.53 ± 0.04	0.68 ± 0.03[c] (128)
Food consumption (g/day/rat)[b]	15.8	15.0 (95)

[a]Doses are converted from % of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), and then ppm intake in food is adjusted using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Mean ± SD, (percentage of control); calculated for this review.
[c]Significantly different from controls ($p \leq 0.001$) by Students *t*-test performed by the study authors.

Source: Hirose et al. (1993).

Table B.12. Average Relative Organ Weights in the Wistar Rat Exposed to Butylated Hydroxytoluene by Diet for 104 Weeks

Parameter (g/100 g bw)	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]		
	0% (0)	**0.25% (184)**	**1% (736)**
Male Rats			
Relative Liver Weight[b]	2.5	4.1 (164)	3.7 (148)
Parameter (g/100 g bw)	**Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]**		
	0% (0)	**0.25% (210)**	**1% (842)**
Female Rats			
Relative Liver Weight[b]	2.8	2.9 (104)	3.5 (125)
Relative Spleen Weight[b]	0.34	0.21(62)	0.23 (68)

[a]Doses were converted from % food to ppm by dividing by 10,000 (1% = 10,000 ppm) and then converted to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Mean, (corresponding percentage of control); calculated for this review.

Source: Hirose et al. (1981).
Notes: Quantitative statistics were not provided for this data from the study and cannot be performed independently due to lack of information.

Table B.13. Selected Hematology and Clinical Chemistry Parameters in the Wistar Rat Exposed to Butylated Hydroxytoluene by Diet for 104 Weeks

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]		
	0% (0)	**0.25% (184)**	**1% (736)**
Male Rats			
Serum Triglycerides (mg/dl)	180	140[b]	137[b]
γ-GTP (mU/dl)	2.8	3.8[c]	4.4[c]
Total Cholesterol (mg/dl)	83.1	94.8[d]	NR
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]**		
	0% (0)	**0.25% (210)**	**1% (842)**
Female Rats			
Red Blood Cells ($x10^4/mm^3$)	585	612[d]	606[d]
Total Cholesterol (mg/dl)	73.4	99.2[c]	112[c]

[a]Doses were converted from % food to ppm by dividing by 10,000 (1% = 10,000 ppm) and then converted to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Significantly different from control ($p \leq 0.01$) using the Student *t*-test, as reported by the study authors.
[c]Significantly different from control ($p \leq 0.001$) using the Student *t*-test, as reported by the study authors.
[d]Significantly different from control ($p \leq 0.05$) using the Student *t*-test, as reported by the study authors.

Source: Hirose et al. (1981).
Notes: NR= Not Reported.

Table B.14. Tumor Incidence in Wistar Rats Administered Butylated Hydroxytoluene by Diet for 104 Weeks

	Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]					
	Males			Females		
Parameter	0% (0)	0.25% (52)	1% (210)	0% (0)	0.25% (54)	1% (215)
Sample size	**26**	**43**	**38**	**32**	**46**	**51**
Liver						
Hyperplastic nodule[c]	2 (7.7)	2 (4.7)	1 (2.6)	0	3 (6.5)	3 (5.9)
Pancreas						
Carcinoma[c]	0	0	1 (2.6)	0	1 (2.2)	4 (7.8)
Islet-cell adenoma[c]	0	1 (2.3)	2 (5.3)	0	0	0
Mammary gland						
Fibro-adenoma[c]	NA	NA	NA	6 (18.8)	8 (17.4)	8 (15.7)
Adenoma[c]	NA	NA	NA	1 (3.4)	1 (2.2)	1 (2.0)
Uterus						
Leiomyoma[c]	NA	NA	NA	1 (3.4)	1 (2.2)	0
Carcinoma[c]	NA	NA	NA	1 (3.1)	2 (4.3)	1 (2.0)
Pituitary gland						
Adenoma[c]	2 (7.7)	3 (7.0)	1 (2.6)	0	6 (13.0)[d]	3 (11.8)
Carcinoma[c]	0	2 (4.7)	5 (13.2)	3 (9.4)	3 (6.5)	7 (13.7)
Adrenal gland						
Adenoma[c]	1 (3.8)	3 (7.0)	0	0	2 (4.3)	1 (2.0)
Carcinoma[c]	0	0	0	0	0	1 (2.0)
Other[c,e]	2 (7.7)	2 (4.7)	4 (10.5)	2 (6.3)	4 (8.7)	3 (11.8)
Total[c]	6 (23.1)	13 (30.2)	10 (26.3)	11 (34.4)	25 (54.3)	25 (49.0)

[a]Doses were converted from % food to ppm by dividing by 10,000 (1% = 10,000 ppm) and then converted to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Doses were converted from adjusted daily doses to human equivalency doses using the following formula:
$Dose_{HED}$ = $Dose_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)^(.25).
[c]Incidence, (corresponding percentage); reported by the study authors; Animals that survived more than 69 weeks were included.
[d]Significantly different from control ($p \leq 0.05$) using the chi-square test, as reported by the study authors.
[e]Other tumors were a malignant lymphoma of the lung and an osteosarcoma in the limb of one male control; a subcutaneous fibroma and a thyroid adenoma in male rats given 0.25% BHT diet; a subcutaneous fibroma, a subcutaneous lipoma, a chronic myelogenous leukemia and a thyroid adenoma in male rats fed 1% BHT diet; and osteosarcoma in the limb and a thyroid adenoma in a female control; a kidney liposarcoma, a subcutaneous rhabdomyosarcoma, a uterine lipoma and a subcutaneous squamous-cell carcinoma in female rats fed 0.25% BHT diet; a rhabdomyosarcoma in the retroperitoneum, an osteosarcoma in a limb and a subcutaneous fibroma in female rats fed 1% BHT diet.

Source: Hirose et al. (1981).
Notes: NA = Not Applicable.

Table B.15. Selected Hematology, Body and Organ Weights in Albino Wistar Male Rats Exposed to Butylated Hydroxytoluene via Diet for 24 Months

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]					
	0% (0)	**0.2% (147)**	**0.5% (368)**	**0.5% (589)[b]**	**0.8% (589)**	**1.0% (736)**
Body-weight gain (g)[c]	367	362 (99)	352 (96)	389 (106)	324 (88)	241 (66)
Brain (% bw)[c]	0.476	0.481 (101)	0.483 (102)	0.478 (100)	0.537 (113)	0.663 (139)
Heart (% bw)[c]	0.397	0.387 (98)	0.388 (98)	0.330 (83)	0.361 (91)	0.366 (92)
Lung (% bw)[c]	1.12	1.34 (89)	1.00 (89)	1.04 (93)	0.781 (68)	0.871 (78)
Kidney (% bw)[c]	0.830	0.884 (107)	0.908 (113)	0.804 (97)	0.893 (108)	0.897 (108)
Spleen (% bw)[c]	0.402	0.278 (69)	0.376 (94)	0.303 (75)	0.291 (72)	0.316 (79)
Liver (% bw)[c]	3.85	4.04 (105)	4.55 (118)	4.51 (117)	4.87 (127)	5.85 (152)
Testes (% bw)[c]	0.774	0.804 (104)	0.758 (98)	0.800 (103)	0.862 (111)	1.01 (131)
Erythrocytes (millions/mm^3)[d]	8.9 [7.3 to 10.4]	9.2 [7.0 to 12.1]	9.8 [6.7 to 13.7]	9.0 [6.8 to 12.6]	9.4 [6.9 to 12.0]	NR
Leucocytes (thousands/mm^3)[d]	15.7 [5.5 to 29.7]	16.4 [7.6 to 32.5]	12.3 [5.6 to 19.7]	15.8 [9.4 to 23.9]	16.4 [8.2 to 32.0]	NR
Hemoglobin (g/100 mL)[d]	14.8 [13.5 to 16.4]	14.6 [13.6 to 16.2]	14.5 [13.6 to 15.5]	14.5 [13.2 to 15.7]	14.5 [12.7 to 16.0]	NR

[a]Doses were converted from % food to ppm by dividing by 10,000 (1% = 10,000 ppm) and then converted to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]0.5% BHT dissolved in lard, heated for 30 minutes at 150 °C and administered in feed.
[c]Mean, (corresponding percentage of control); calculated for this review.
[d]Mean [90% values].

Source: Deichmann et al. (1955d).
Notes: Quantitative statistics not provided for this data from the study and cannot be performed independently due to lack of information. NR = Not Reported.

Table B.16. Selected Hematology, Body and Organ Weights in the Albino Wistar Female Rat Exposed to Butylated Hydroxytoluene via Diet for 24 Months

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]					
	0% (0)	**0.2% (168)**	**0.5% (421)**	**0.5% (421)[b]**	**0.8% (673)**	**1.0% (842)**
Body-weight gain (g)[c]	237	224 (95)	241 (102)	243 (103)	228 (96)	172 (73)
Brain (% bw)[c]	0.616	0.681 (111)	0.630 (102)	0.606 (98)	0.608 (99)	0.765 (124)
Heart (% bw)[c]	0.480	0.531 (111)	0.475 (99)	0.469 (98)	0.468 (98)	0.551 (115)
Lung (% bw)[c]	0.996	0.741 (74)	0.768 (77)	1.04 (104)	1.02 (102)	1.20 (121)
Kidney (% bw)[c]	0.910	1.01 (111)	0.854 (94)	0.920 (101)	0.778 (85)	0.897 (99)
Spleen (% bw)[c]	0.290	0.290 (100)	0.257 (89)	0.308 (106)	0.249 (86)	0.239 (82)
Liver (% bw)[c]	4.57	4.25 (93)	4.10 (90)	5.20 (114)	5.19 (114)	5.32 (116)
Erythrocytes (millions/mm^3)[d]	8.2 [6.6 to 9.2]	8.2 [6.8 to 9.9]	8.7 [7.2 to 10.5]	8.1 [6.9 to 9.1]	8.9 [7.6 to 10.8]	9.6 [7.9 to 11.3]
Leucocytes (thousands/mm^3)[d]	13.4 [7.7 to 22.6]	9.6 [5.4 to 18.8]	10.1 [7.0 to 15.8]	10.8 [5.3 to 15.1]	10.4 [6.2 to 17.5]	9.9 [6.0 to 16.0]
Hemoglobin (g/100 mL)[d]	14.5 [12.2 to 16.8]	14.1 [13.0 to 15.1]	14.4 [12.8 to 15.3]	15.0 [13.4 to 16.1]	14.6 [12.3 to 16.8]	14.3 [13.0 to 15.5]

[c]Mean, (corresponding percentage of control); calculated for this review.
[d]Mean [90% values].
[b]0.5% BHT dissolved in lard, heated for 30 minutes at 150 °C and administered in feed.
[a]Doses were converted from % food to ppm by dividing by 10,000 (1% = 10,000 ppm) and then converted to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).

Source: Deichmann et al. (1955d).
Notes: Quantitative statistics not provided for this data from the study and cannot be performed independently due to lack of information.

Table B.17. Survival of Male and Female F344 Rats after Oral Exposure to Butylated Hydroxytoluene for 105 Weeks

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]		
	0 ppm (0)	**3000 ppm (237)**	**6000 ppm (474)**
Male Rats			
Sample size	20	50	50
Survival[b]	13 (65)	39 (78)	36 (72)
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]**		
	0 ppm (0)	**3000 ppm (275)**	**6000 ppm (550)**
Female Rats			
Sample size	20	50	50
Survival[b]	13 (65)	37 (74)	39 (78)

[a]Doses were converted from ppm to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Number of animals surviving to the end of the study period, (corresponding percentage).

Source: NCI (1979c).

Table B.18. Incidence of Nonneoplastic Focal Alveolar Histiocytosis in Male and Female Rats after Oral Exposure to Butylated Hydroxytoluene for 105 Weeks

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]		
	0 ppm (0)	**3000 ppm (237)**	**6000 ppm (474)**
Male Rats			
Sample size	20	49	49
Focal alveolar histiocytosis[b]	1 (5)	4 (8)	7 (14)
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-day)[b]**		
	0 ppm (0)	**3000 ppm (275)**	**6000 ppm (550)**
Female Rats			
Sample size	18	48	49
Focal alveolar histiocytosis[b]	2 (11)	12 (25)	21 (43)[c]

[a]Doses converted to adjusted daily dose using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days)
[b]Number of animals with observation, (corresponding percentage)
[c]Statistically significant ($p < 0.05$) from independent χ^2 test performed for this analysis

Source: NCI (1979c).

Table B.19. Selected Incidence of Neoplasms in Male and Female F344 Rats After Oral Exposure to Butylated Hydroxytoluene for 105 Weeks

Parameter		Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]		
		0 ppm (0)	**3000 ppm (64)**	**6000 ppm (129)**
Male Rats				
Lung	Squamous cell carcinoma, metasta[c]	0/20 (0)	1/49 (2)	0/49 (0)
	Alveolar/bronchiolar adenoma[c]	0/20 (0)	0/49 (0)	2/49 (4)
	Alveolar/bronchiolar carcinoma[c]	1/20 (5)	1/49 (2)	1/49 (2)
Liver	Bile duct carcinoma[c]	0/20 (0)	0/48 (0)	1/48 (2)
	Neoplastic nodule[c]	0/20 (0)	1/48 (2)	1/48 (2)
	Hepatocellular carcinoma[c]	0/20 (0)	1/48 (2)	1/48 (2)
Small intestine	Lipoma[c]	0/18 (0)	0/48 (0)	1/48 (2)
Kidney	Nephroblastoma[c]	0/20 (0)	1/49 (2)	0/48 (0)
Urinary bladder	Transitional-cell carcinoma[c]	0/20 (0)	1/47 (2)	0/46 (0)
Testis	Interstitial-cell tumor[d,c]	15/20 (75)	42/49 (86)	32/49 (65)
Parameter		**Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]**		
		0 ppm (0)	**3000 ppm (66)**	**6000 ppm (132)**
Female Rats				
Lung	Alveolar/bronchiolar adenoma[c]	1/18 (6)	2/48 (4)	0/49 (0)
	Alveolar/bronchiolar carcinoma[c]	0/18 (0)	1/48 (2)	1/49 (2)
Pituitary gland	Adenoma, NOS[d,c]	8/18 (44)	9/48 (19)	5/49 (10)
Uterus	Carcinoma, NOS[c]	0/17 (0)	0/49 (0)	1/49 (2)
	Endometrial stromal polyp[c]	2/17 (12)	8/49 (16)	6/49 (12)

[a]Doses were converted from ppm to adjusted daily doses in mg/kg-day using the following formula: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Doses converted to human equivalency doses using: HED = $NOAEL_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)^(.25).
[c]Number of animals with observation/ total number examined () –corresponding percentage; reported by study authors.
[d]Significant negative dose-related trend by Cochran-Armitage test performed by authors.

Source: NCI (1979c).

Table B.20. Selected Tumor Incidence in Male and Female F344 Rats after Oral Exposure to Butylated Hydroxytoluene for 105 weeks

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]		
	0 ppm (0)	**3000 ppm (64)**	**6000 ppm (129)**
Male Rats			
Animals in study	20	50	50
Animals examined histopathologically	20	49	49
Animals with primary tumors[c]	19 (95)	46 (94)	44(90)
Animals with benign tumors[c]	18 (90)	45 (92)	41 (84)
Animals with malignant tumors[c]	9 (45)	19 (39)	20 (41)
Parameter	**Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]**		
	0 ppm (0)	**3000 ppm (66)**	**6000 ppm (132)**
Female Rats			
Animals in study	20	50	50
Animals examined histopathologically	18	49	50
Animals with primary tumors[c]	12 (67)	36 (73)	26 (52)
Animals with benign tumors[c]	11 (61)	27 (55)	18 (36)
Animals with malignant tumors[c]	2 (11)	16 (33)	9 (18)

[a]Doses were converted from ppm to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Doses converted to human equivalency doses using: HED = $NOAEL_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)^(.25).
[c]Number of animals with observation, (corresponding percentage); calculated for this review.

Source: NCI (1979c).

Table B.21. Reproduction Data for F0 Wistar Rats Treated with Butylated Hydroxytoluene in Diet

Parameter		Exposure Group, mg/kg-day			
		0	**25**	**100**	**500**
No. of rats/dose group	Females	40	29	30	44
	Males	39	29	30	44
Gestation rate (%)		88	95	93	95
No. of pups/litter	Mean	10.9	9.6	10.3	9.1[a]
	After standardization	8.0	8.0	8.0	7.9
	At weaning	7.9	8.0	7.7	7.8
Body weight (g) of pups[b]	At birth	5.9	5.9 (100)	5.7[c] (97)	5.7 (97)
	At Weaning	42.4	40.4[c] (95)	39.7[d] (94)	25.3[d] (60)

[a]Armitage-Cochran test for linear trend in proportions of litters with ten or more pups ($p \leq 0.001$) conducted by the study authors.
[b]Average of mean pup weight/litter, (percentage of control); calculated for this review.
[c]Statistically significant by the ($p \leq 0.05$) Students *t*-test conducted by the study authors.
[d]Statistically significant by the ($p \leq 0.001$) Students *t*-test conducted by the study authors.

Source: Olsen et al. (1986).

Table B.22. Mean Body Weights of F1 Adult Wistar Rats Treated with Butylated Hydroxytoluene in Diet for 141−144 Weeks[a]

Exposure Group, mg/kg-day	No. of Animals	Mean Body Weights (g) at Week						
		5	7	9	15	34	90	138
Male Rats								
0	100	105	186	243	357	471	575	487
25	80	103 (98)	181[b] (97)	244 (100)	350 (98)	453[c] (96)	550[c] (96)	450[b] (92)
100	80	97[d] (92)	181[b] (97)	243 (100)	346[b] (97)	447[d] (95)	516[d] (90)	433[c] (89)
250[e]	99	83[d] (79)	150[d] (81)	216[d] (89)	301[d] (84)	385[d] (82)	459[d] (80)	413[d] (85)
Exposure Group, mg/kg-day	**No. of Animals**	**Mean Body Weights (g) at Week**						
		5	**7**	**9**	**15**	**34**	**90**	**138**
Female Rats								
0	100	86	140	176	231	277	344	313
25	79	86 (100)	139 (99)	176 (100)	227 (98)	268[c] (97)	343 (100)	312 (100)
100	80	89[b] (103)	139 (99)	176 (100)	226[b] (98)	260[d] (94)	319[d] (93)	305 (97)
250[e]	99	68[d] (79)	122[d] (87)	159[d] (90)	208[d] (90)	247[d] (89)	288[d] (84)	281[c] (90)

[a]Average of mean body weights, (percentage of control); calculated for this review.
[b]Armitage-Cochran test for linear trend in proportions of litters with ten or more pups ($p \leq 0.001$) conducted by the study authors.
[c]Statistically significant by the ($p \leq 0.05$) Students *t*-test conducted by the study authors.
[d]Statistically significant by the ($p \leq 0.001$) Students *t*-test conducted by the study authors.
[e] Rats in the high dose group were born to dams that were exposed to 500 mg/kg/day during gestation and lactation but that the dose was reduced to 250 mg/kg/day in F1s that were exposed through feed after weaning.

Source: Olsen et al. (1986).

Table B.23. Mortality in F1 Adult Wistar Rats Treated with Butylated Hydroxytoluene in Diet for 141−144 Weeks

Exposure Group, mg/kg-day	No. of Animals	No. of Rats Dying During Specified Weeks[a]							
		0−90	91−104	105−113	114−118	119−126	127−132	133−140	Total[b]
Males									
0	100	20 (20)	10 (10)	13 (13)	8 (8)	11 (11)	10 (10)	12 (12)	84 (84)
25	80	8 (10)	11 (14)	6 (8)	3 (4)	13 (16)	11 (14)	8 (10)	60 (75)
100	80	8 (10)	12 (15)	3 (4)	2 (3)	10 (13)	7 (9)	11 (14)	53 (66)
250	99	7 (9)	7 (19)	6 (8)	4 (5)	8 (10)	13 (16)	10 (13)	55 (56)
Females									
0	100	16 (16)	15 (15)	18 (18)	8 (8)	11 (11)	7 (7)	8 (8)	83 (83)
25	79	10 (13)	9 (11)	4 (5)	6 (8)	13 (16)	10 (13)	8 (10)	60 (76)
100	80	5 (6)	17 (21)	5 (6)	5 (6)	7 (9)	9 (11)	11 (14)	59 (74)
250	99	9 (9)	5 (5)	11 (11)	12 (12)	8 (8)	5 (5)	10 (10)	60 (61)

[a]Number of dead animals, (percentage of total animals); calculated for this review.
[b]Calculated for this review from data reported in the study.

Source: Olsen et al. (1986).

Table B.24. Serum Chemistry of F1 Adult Wistar Rats Treated with Butylated Hydroxytoluene in Diet for 141−144 Weeks

Serum Chemistry Parameter	Exposure Group, mg/kg-day	Conc. (nmol/liter serum) at week (of age)			
		9	19	43	108
Male Rats					
Free Cholesterol[a]	0	0.53 ± 0.02	0.64 ± 0.04	0.72 ± 0.03	1.13 ± 0.10
	250	0.64 ± 0.04[b] (121)	0.68 ± 0.03 (106)	0.66 ± 0.03 (92)	1.10 ± 0.08 (97)
Total Cholesterol[a]	0	2.05 ± 0.09	2.31 ± 0.16	2.84 ± 0.13	4.14 ± 0.38
	250	2.27 ± 0.11 (110)	2.26 ± 0.10 (98)	2.45 ± 0.08[b] (86)	3.82 ± 0.25 (92)
Phospholipids[a]	0	2.82 ± 0.20	2.45 ± 0.07	2.93 ± 0.13	2.95 ± 0.21
	250	2.52 ± 0.02 (89)	2.35 ± 0.08 (96)	2.51 ± 0.09[b] (86)	2.83 ± 0.20 (96)
Triglycerides[a]	0	NR	1.67 ± 0.20	1.85 ± 0.22	1.76 ± 0.21
	250	NR	0.75 ± 0.08[d] (45)	0.97 ± 0.12[c] (52)	1.24 ± 0.17 (70)
Serum Chemistry Parameter	**Exposure Group, mg/kg-day**	**Conc. (nmol/liter serum) at week (of age)**			
		9	**19**	**43**	**108**
Female Rats					
Free Cholesterol[a]	0	0.58 ± 0.02	0.68 ± 0.03	0.75 ± 0.03	0.93 ± 0.06
	250	0.82 ± 0.04[d] (141)	0.83 ± 0.04[c] (122)	0.92 ± 0.05[c] (123)	0.81 ± 0.04 (87)
Total Cholesterol[a]	0	2.02 ± 0.10	2.12 ± 0.10	2.72 ± 0.13	3.21 ± 0.19
	250	2.63 ± 0.11[d] (131)	2.76 ± 0.11[d] (130)	2.97 ± 0.15 (109)	2.81 ± 0.15 (88)
Phospholipids[a]	0	2.74 ± 0.12	2.53 ± 0.08	3.21 ± 0.11	3.07 ± 0.22
	250	2.89 ± 0.07 (105)	2.99 ± 0.09[d] (118)	3.33 ± 0.12 (104)	2.50 ± 0.14[b] (81)
Triglycerides[a]	0	NR	1.28 ± 0.15	2.02 ± 0.17	3.42 ± 0.38
	250	NR	0.97 ± 0.08 (76)	1.10 ± 0.12[d] (55)	1.20 ± 0.10[d] (35)

[a]Means ± SE, (corresponding percentage of control); calculated for this review.
[b]Statistically significant by the ($p \leq 0.05$) Students *t*-test conducted by the study authors.
[c]Statistically significant by the ($p \leq 0.01$) Students *t*-test conducted by the study authors.
[d]Statistically significant by the ($p \leq 0.001$) Students *t*-test conducted by the study authors.

Source: Olsen et al. (1986).
Notes: Notes: NR= Not Reported.

Table B.25. Incidence of Hepatocellular Nodular Hyperplasia, Hepatocellular Adenomas and Carcinomas in F1 Adult Wistar Rats Treated with Butylated Hydroxytoluene in Diet for 141−144 Weeks

Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]	No. of Animals	No. of Rats with Tumors			Total Tumors[d]
		Nodular Hyperplasia[c]	Adenoma[c]	Carcinoma[c]	
Male Rats					
0 (0)	100	2 (2)	1 (1)	1 (1)	2 (2)
25 (7.1)	80	0 (0)	1 (1)	0 (0)	1 (1)
100 (28)	80	2 (2)	5 (6)	1 (1)	6 (8)
250 (69)	99	2 (2)	18[e] (18)	8[f] (8)	26 (26)[g]
Exposure Group (HED, mg/kg-day)[b]	**No. of Animals**	**No. of Rats with Tumors**			**Total Tumors[d]**
		Nodular Hyperplasia[a]	**Adenoma[a]**	**Carcinoma[a]**	
Female Rats					
0 (0)	100	2 (2)	2 (2)	0 (0)	2 (2)
25 (6.4)	79	0 (0)	3(4)	0 (0)	3 (4)
100 (25)	80	4 (5)	6 (8)	0 (0)	6 (8)
250 (62)	99	5 (5)	12[h] (12)	2[i] (2)	14 (14)[g]

[a]Doses were converted from ppm to adjusted daily doses in mg/kg-day using the following formula: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Doses converted to human equivalency doses using:
$HED = NOAEL_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)^(.25).
[c]Number of animals with tumors, (corresponding percentage); calculated for this review.
[d]Total tumors equals the sum of adenoma and carcinoma combined.
[e]Overall test for heterogeneity, $p < 0.05$; Test for trend, $p < 0.01$.
[f]Overall test for heterogeneity, $p < 0.001$; Test for trend, $p < 0.001$.
[g]Statistically significant ($p < 0.05$) difference from controls by Chi-square test.
[h]Overall test for heterogeneity, not significant; Test for trend, $p < 0.05$.
[i]Overall test for heterogeneity, not significant; Test for trend, not significant.

Source: Olsen et al. (1986).

Table B.26. Weeks During which Hepatocellular Adenomas and Carcinomas were Detected in F1 Adult Wistar Rats Treated with Butylated Hydroxytoluene in Diet for 141−144 Weeks

Dose Group (HED, mg/kg-day)[a]	Age (week) at which tumors were detected	
	Adenomas	Carcinomas
Male Rats		
0 mg/kg-day (0)	133	117
25 mg/kg-day (7.1)	119	
100 mg/kg-day (28)	136, 139, 141, 143, 143	141
250 mg/kg-day (69)	115, 119, 125, 127, 138, 141, 141, 141, 141, 141, 141, 142, 142, 142, 142, 142, 143, 144	132, 141, 142, 142, 143, 143, 143, 143
Dose Group (HED, mg/kg-day)[a]	**Age (week) at which tumors were detected**	
	Adenomas	**Carcinomas**
Female Rats		
0 mg/kg-day (0)	117, 117	
25 mg/kg-day (6.4)	132, 134, 143	
100 mg/kg-day (25)	125, 129, 136, 142, 142, 143	
250 mg/kg-day (62)	134, 135, 140, 140, 141, 141, 142, 142, 142, 142, 143, 143	141, 143

[a]Doses converted to human equivalency doses using:
HED = Dose × (Body Weight Animal ÷ Body Weight Human)^(.25).

Source: Olsen et al. (1986).

Table B.27. Number of Tumor-Bearing F1 Adult Wistar Rats Treated with Butylated Hydroxytoluene in Diet (Excludes Animals with Hepatocellular Tumors) for 141−144 Weeks[a]

	Males				Females			
Dose Group (HED, mg/kg-day)[b]	**0 mg/kg-day (0)**	**25 mg/kg-day (7.1)**	**100 mg/kg-day (28)**	**250 mg/kg-day (69)**	**0 mg/kg-day (0)**	**25 mg/kg-day (6.4)**	**100 mg/kg-day (25)**	**250 mg/kg-day (62)**
No. of Animals	**100**	**80**	**80**	**99**	**100**	**79**	**80**	**99**
Total tumor-bearing rats	81 (81)	69 (86)	70 (88)	86 (87)	85 (85)	70 (89)	73 (91)	90 (91)
Animals with malignant tumors	36 (36)	28 (35)	32 (40)	48 (49)	21 (21)	27 (37)	17 (21)	35 (35)
Benign tumors	66 (66)	55 (69)	54 (68)	70 (71)	77 (77)	67 (85)	69 (86)	78 (79)
One tumor	38 (38)	43 (54)	42 (53)	37 (37)	38 (38)	23 (26)	30 (38)	36 (36)
Two tumors	22 (22)	15 (19)	17 (21)	24 (24)	27 (27)	29 (37)	24 (30)	25 (25)
Multiple tumors	21 (21)	11 (14)	11 (14)	25 (25)	20 (20)	18 (23)	19 (24)	29 (29)

[a]Number of animals with tumors, () –corresponding percentage; calculated for this review.
[b]Doses converted to human equivalency doses using: HED = Dose × (Body Weight Animal ÷ Body Weight Human)^(.25).

Source: Olsen et al. (1986).

Table B.28. Occurrence of Nonneoplastic Lesions in F1 Adults Wistar Rats Treated with Butylated Hydroxytoluene in Diet for 141−144 Weeks

	Males				Females			
Dose Group (HED, mg/kg-day)[a]	**0 mg/kg-day (0)**	**25 mg/kg-day (7.1)**	**100 mg/kg-day (28)**	**250 mg/kg-day (69)**	**0 mg/kg-day (0)**	**25 mg/kg-day (6.4)**	**100 mg/kg-day (25)**	**250 mg/kg-day (62)**
No. of Animals	**100**	**80**	**80**	**99**	**100**	**79**	**80**	**99**
Heart								
Fibrosis	72	40	44	36	43	22	25	9
Endocardiosis	1	4	1	2	-	-	-	2
Calcification	-	-	2	-	2	1	1	3
Liver								
Fibrosis	-	-	1	-	1	4	1	3
Angiectasis	2	4	3	6	2	6	4	5
Eosinophilic necrosis	-	-	-	1	-	1	-	1
Basophilic areas	3	3	2	1	9	2	7	1
Focal cellular enlargement	6	7	14	8	1	7	11	16
Cysts	1	1	6	17	7	2	1	9
Fatty metamorphosis	11	10	3	3	3	3	1	-
Peliosis	2	2	4	4	1	2	4	-
Bile-duct proliferation	1	2	5	12	5	5	2	4
Hemorrhage	2	-	-	1	-	-	1	-

[a]Doses converted to human equivalency doses using:
HED = Dose × (Body Weight Animal ÷ Body Weight Human)^(.25).

Source: Olsen et al. (1986).
Notes: Quantitative statistics not provided for this data from the study and cannot be performed independently due to lack of information.

Table B.29. Survival of Male and Female B6C3F$_1$ Mice after Oral Exposure to Butylated Hydroxytoluene for 107 Weeks[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[b]		
	0 ppm (0)	**3000 ppm (515)**	**6000 ppm (1029)**
Male Mice			
Sample size	20	50	50
Survival	12(60)	43 (86)	46 (92)
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-day)[b]**		
	0 ppm	**3000 ppm (518)**	**6000 ppm (1037)**
Female Mice			
Sample size	20	50	50
Survival	17 (85)	41 (82)	45 (90)

[a]Number of animals surviving to the end of the study (corresponding percentage); reported by study authors.
[b]Doses were converted from ppm to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).

Source: NCI (1979d).

Table B.30. Selected Nonneoplastic and Neoplastic Pathology Findings in Male and Female B6C3F$_1$ Mice after Oral Exposure to Butylated Hydroxytoluene for 107 Weeks

Parameter		Exposure group (Human Equivalency Dose, mg/kg-day)[a,b]		
Male Mice		**0 ppm (0)**	**3000 ppm (78)**	**6000 ppm (155)**
Liver	Hepatocytomegaly[c]	0/20 (0)	9/48 (19)[d]	20/49 (41)[d]
	Hepatocellular adenoma[c]	2/20 (10)	11/48 (23)	7/49 (14)
	Hepatocellular carcinoma[e,c]	9/20 (45)	12/48 (25)	6/49 (12)[d]
	Angiosarcoma[c]	1/20 (5)	0/48 (0)	1/49 (2)
	Peliosis[c]	0/20 (0)	34/48 (71)[d]	43/49 (88)[d]
	Hepatocellular degeneration/necrosis[c]	2/20 (10)	34/48 (71)[d]	45/49 (92)[d]
	Cytoplasmic vacuolation[c]	3/20 (15)	20/48 (42)[d]	22/49 (45)[d]
Lung	Alveolar/bronchiolar carcinoma[c]	5/20 (25)	12/50 (24)	7/49 (14)
	Adenoma[c]	2/20 (10)	9/50 (18)	10/49 (20)
Eye/lacrimal gland	Adenoma[f,c]	0/20 (0)	0/50 (0)	4/50 (8)
Parameter		**Exposure group (Human Equivalency Dose, mg/kg-day)[a,b]**		
Female Mice		**0 ppm (0)**	**3000 ppm (78)**	**6000 ppm (155)**
Liver	Hepatocytomegaly[c]	0/20 (0)	1/46 (2)	1/49 (2)
	Hepatocellular adenoma[c]	0/20 (0)	3/46 (7)	2/49 (4)
	Hepatocellular carcinoma[c]	1/20 (5)	1/46 (2)	3/49 (6)
	Angiosarcoma[c]	1/20 (5)	1/46 (2)	1/49 (2)
	Peliosis[c]	0/20 (0)	0/46 (0)	0/49 (0)
	Hepatocellular degeneration/necrosis[c]	0/20 (0)	0/46 (0)	0/49 (0)
	Cytoplasmic vacuolation[c]	0/20 (0)	0/46 (0)	0/49 (0)
Lung	Alveolar/bronchiolar carcinoma[c]	1/20 (5)	4/46 (9)[g]	4/50 (8)
	Adenoma[c]	0/20 (0)	12/46 (26)[g,d]	3/50 (6)
Eye/lacrimal gland	Adenoma[f,c]	0/20 (0)	2/46 (4)	0/50 (0)

[a]Doses were converted from ppm to adjusted daily doses in mg/kg-day using the following formula:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Doses converted to human equivalency doses using:
HED = $Dose_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)$^{(0.25)}$.
[c]NOS = Number of animals with observation ÷ total number of animals examined () –corresponding percentage.
[d]Statistically significant ($p < 0.05$) from independent χ^2 test performed for this analysis.
[e]Significant negative dose-related trend by the Cochran-Armitage test performed by study authors.
[f]Significant dose-related trend by the Cochran-Armitage test performed by study authors.
[g]Significant difference between incidence of alveolar/bronchiolar carcinoma or adenoma in low dose females and controls by Fisher exact test performed by study authors.

Source: NCI (1979d).

Table B.31. Selected Tumor Incidence in Male and Female B6C3F$_1$ Mice after Oral Exposure to Butylated Hydroxytoluene for 107 Weeks

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]		
	0 ppm (0)	3000 ppm (78)	6000 ppm (155)
Male Mice			
Animals in study	20	50	50
Animals examined histopathologically	20	50	49
Animals with primary tumors (%)[c]	17 (85)	39 (78)	32 (65)
Animals with benign tumors (%)[c]	4 (20)	20 (40)	19 (39)
Animals with malignant tumors (%)[c]	16 (64)	32 (64)	19 (39)[d]
Parameter	**Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]**		
	0 ppm (0)	**3000 ppm (78)**	**6000 ppm (155)**
Female Mice			
Animals in study	20	50	50
Animals examined histopathologically	20	46	50
Animals with primary tumors (%)[c]	14 (70)	32 (70)	23 (46)
Animals with benign tumors (%)[c]	2 (10)	22 (48)[d]	10 (20)
Animals with malignant tumors (%)[c]	13 (65)	16 (35)[d]	17 (34)[d]

[a]Doses converted to adjusted daily dose using the following formula: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷Total Days).
[b]Doses converted to human equivalency doses using: HED = $Dose_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)$^{(0.25)}$.
[c]Number of animals with observation ÷ total number of animals examined, () –corresponding percentage.
[d]Statistically significant (p <0.05) from independent χ^2 test performed for this analysis.

Source: NCI (1979d).

Table B.32. Average Final Body and Liver Weights in B6C3F$_1$ Male Mice Without Tumors Exposed to Butylated Hydroxytoluene by Diet for 104 Weeks

Tumor Type	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]		
	0% (0)	1% (1640)	2% (3480)
Final body weight (g)[b]	30.2 ± 5.9	31.3 ± 5.4	28.8 ± 5.1
Liver weight (g)[b]	1.72 ± 0.31	2.42 ± 0.43[c]	2.40 ± 0.73[d]
Relative liver weight (% of body weight)[b]	5.8 ± 0.7	7.9 ± 1.4[c]	8.2 ± 1.9[c]

[a]Doses were converted from % food, to ppm by dividing by 10,000 (1% = 10,000 ppm). Adjusted daily doses were calculated using the following equation: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷Body Weight) × (Days Dosed ÷ Total Days).
[b]Means ± SD.
[c]Significantly different from controls at ($p \leq 0.001$) by Student's t- test conducted by study authors.
[d]Significantly different from controls at ($p \leq 0.01$) by Student's t- test conducted by study authors.

Source: Inai et al. (1988).

Table B.33. Incidences of Hepatocellular Lesions in B6C3F$_1$ Male Mice Exposed to Butylated Hydroxytoluene by Diet for 104 Weeks

Tumor Type	Exposure Group (Human Equivalency Dose, mg/kg-day)[a,b]		
	0% (0)	1% (249)	2% (529)
No. of Foci of Cellular Alteration	1/32 (3)	25/42[c] (60)	42/47[c] (89)
Adenoma	6/32 (19)	16/42 (38)	25/47[c] (53)
Carcinoma	7/32 (22)	11/42 (26)	8/47 (17)
Hemangioma	4/32 (13)	3/42 (7)	1/47 (2)
Angiosarcoma	0/32 (0)	0/42 (0)	1/47 (2)

[a]Doses were converted from % food, to ppm by dividing by 10,000 (1% = 10,000 ppm). Adjusted daily doses were calculated using the following equation: $Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷Body Weight) × (Days Dosed ÷Total Days).
[b]Doses were converted to human equivalency doses using the following formula: HED = $Dose_{ADJ}$ × (Body Weight Animal ÷ Body Weight Human)^(.25).
[c]Significantly different from controls at ($p \leq 0.05$) by chi-square test conducted by study authors.

Source: Inai et al. (1988)

Table B.34. Liver and Body Weights in Wistar Rat Dams Exposed to Butylated Hydroxytoluene via Diet for through Mating, Pregnancy and Lactation

Parameter	Exposure Group			
	0 mg/kg-day	500 mg/kg-day	750 mg/kg-day	1000 mg/kg-day
Number of dams	**8**	**8**	**7**	**9**
Terminal Body weights (g)[a]	294 ± 7	281 ± 11 (96)	263 ± 7[b] (89)	234 ± 6[b] (80)
Liver weight (g)[a]	17.2 ± 1.0	24.7 ± 1.3[b] (144)	24.7 ± 0.6[b] (144)	22.6 ± 1.0[b] (131)
Relative liver weight (liver/body weight %)[a]	5.81 ± 0.22	8.77 ± 0.25[b] (151)	9.39 ± 0.23[b] (162)	9.66 ± 0.29[b] (166)

[a]Means ± SE, ()–corresponding percentage of control calculated for this review.
[b]Significantly different from control ($p \leq 0.05$) by Students *t*-test performed by study authors.

Source: McFarlane et al. (1997a).

Table B.35. Litter Size, Survival, Body Weights, and Liver Weight of Wistar Rat Pups (F1) Exposed to Butylated Hydroxytoluene in Gestation, Lactation

Parameter	Exposure Group			
	0 mg/kg-day	**500 mg/kg-day**	**750 mg/kg-day**	**1000 mg/kg-day**
Number of dams	**8**	**9**	**9**	**9**
Pups/litter[a]	11.5 ± 1.0	11.4 ± 0.8 (99)	11.0 ± 0.7 (96)	10.0 ± 1.0 (87)
Dead or dying pups (within 4 days of birth)/ total no.	0/92	2/103	0/99	1/90
Litter weight at birth (g)[a]	77.3 ± 5.0	74.0 ± 3.6 (96)	68.1 ± 3.2 (88)	64.1 ± 6.3 (83)
Pup weight at birth (g)[a]	6.83 ± 0.21	6.60 ± 0.28 (97)	6.24 ± 0.16 (91)[b]	6.46 ± 0.12 (95)
Body weight of pups before reduction (g)[a]	53.9 ± 0.4	35.6 ± 2.0 (66)[b]	26.0 ± 0.9 (48)[b]	21.9 ± 0.4 (41)[b]
Body weight of pups after reduction (g)[a]	53.4 ± 0.7	33.7 ± 0.6 (63)[b]	26.5 ± 0.7 (50)[b]	21.5 ± 0.3 (40)[b]
Liver weight of pups (g)[a]	2.18 ± 0.08	1.57 ± 0.15 (72)	1.02 ± 0.09 (47)[b]	0.90 ± 0.09 (41)[b]
Relative liver weight of pups (g)[a]	4.02 ± 0.07	3.85 ± 0.20 (96)	3.95 ± 0.12 (98)	3.95 ± 0.24 (98)

[a]Means ± SE ()–corresponding percentage of control calculated for this review.
[b]Significantly different from control ($p \leq 0.05$) by Students *t*-test performed by study authors.

Source: McFarlane et al. (1997a).

Table B.36. Liver and Body Weights in Wistar Rat Dams Exposed to Butylated Hydroxytoluene in Diet through Gestational Day 19−20

Parameter	Exposure Group			
	0 mg/kg-day	**25 mg/kg-day**	**100 mg/kg-day**	**500 mg/kg-day**
Terminal Body weights (g)[a,b]	292 ± 16	315 ± 10 (108)	309 ± 12 (106)	324 ± 11 (111)
Liver weight (g)[a]	14.56 ± 1.34	13.73 ± 0.70 (94)	14.45 ± 0.64 (99)	17.18 ± 1.16 (118)
Relative liver weight (liver/body weight %)[a,b]	4.97 ± 0.26	4.36 ± 0.15 (88)	4.68 ± 0.08 (94)	5.28 ± 0.24 (106)

[a]Means ± SE ()–corresponding percentage calculated for this review.
[b]Weight of dams excluding the weight of fetuses and associated tissues.

Source: McFarlane et al. (1997b).

Table B.37. Body Weights, Liver Weights and Enzyme Levels of Lactating and Nonlactating Female Wistar Rats Exposed to Butylated Hydroxytoluene via Diet

Parameter	Exposure Group			
	0 mg/kg-day/ nonlactating	**500 mg/kg-day/ nonlactating**	**0 mg/kg-day/ lactating**	**500 mg/kg-day/ lactating**
Body weight (g)[a]	263 ± 5	281 ± 8 (107)	306 ± 10	317 ± 14 (104)
Liver weight (g)[a]	9.15 ± 0.61	13.23 ± 0.62[b] (145)	16.95 ± 0.28	23.89 ± 1.16[b] (141)
Liver/ body-weight ratio (g)[a]	3.47 ± 0.19	4.70 ± 0.11[b] (135)	5.56 ± 0.17	7.53 ± 0.26[b] (135)
Glucose 6-phosphatase (nmol/min/mg protein)[a]	29 ± 2	22 ± 3 (76)	30 ± 2	14 ± 1[b] (47)
Total glutathione (µmol/mg cytosolic protein)[a]	0.060 ± 0.005	0.066 ± 0.003 (110)	0.051 ± 0.002	0.020 ± 0.001[b] (39)
Glutathione *S*-transferase (µmol/min/mg protein)[a]	0.94 ± 0.18	2.02 ± 0.12[b] (215)	1.00 ± 0.10	2.94 ± 0.22[b] (294)
Cytochrome P-450 (pmol/mg protein)[a]	0.339 ± 0.014	0.558 ± 0.070[b] (165)	0.273 ± 0.010	0.461 ± 0.030 (169)
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)[a]	10.03 ± 1.41	9.66 ± 0.78 (96)	4.37 ± 0.45	3.63 ± 0.58 (83)
Pentoxyresorufin *O*-dependentylse (pmol/min/mg protein)[a]	1.55 ± 0.15	100.16 ± 5.79[b] (6462)	2.45 ± 0.11	207.0 ± 24.6[b] (8449)

[a]Means ± SE, (corresponding percentage of corresponding nonlactating or lactating control); calculated for this review.
[b]Significantly different from control ($p \leq 0.05$) by Students *t*-test performed by study authors.

Source: McFarlane et al. (1997b).

Table B.38. Litter Size, Body Weights, Liver Weights and Enzyme Levels of Wistar Rat Pups (F1) Exposed to Butylated Hydroxytoluene via Gestation (GD 19−20)

Parameter	Exposure Group			
	0 mg/kg-day	**25 mg/kg-day**	**100 mg/kg-day**	**500 mg/kg-day**
Pups/litter[a]	11.3 ± 0.8	11.0 ± 1.1 (97)	11.2 ± 0.8 (99)	9.9 ± 1.2 (88)
Fetus weight (g)[a]	2.94 ± 0.12	2.86 ± 0.13 (97)	2.34 ± 0.07[b] (80)	2.71 ± 0.09 (92)
Liver weight of fetuses (g)[a]	0.24 ± 0.01	0.26 ± 0.01 (108)	0.20 ± 0.01 (83)	0.23 ± 0.02 (96)
Relative liver weight of fetuses (g)[a]	8.08 ± 0.27	8.43 ± 0.24 (104)	8.43 ± 0.27 (104)	8.48 ± 0.29 (105)
Glucose 6-phosphatase (nmol/min/mg protein)[a]	2.43 ± 0.75	2.59 ± 0.36 (107)	1.42 ± 0.08[b] (58)	2.04 ± 0.13 (84)
Total glutathione (µmol/mg cytosolic protein)[a]	0.064 ± 0.005	0.065 ± 0.008 (102)	0.074 ± 0.005 (116)	0.072 ± 0.008 (113)
Glutathione *S*-transferase (µmol/min/mg protein)[a]	0.187 ± 0.025	0.226 ± 0.023 (121)	0.195 ± 0.010 (104)	0.212 ± 0.010 (113)
Epoxide hydrolase (nmol/min/mg protein)[a]	1.65 ± 0.47	1.37 ± 0.24 (83)	1.25 ± 0.26 (76)	1.32 ± 0.18 (80)

[a]Means ± SE; (corresponding percentage of control); calculated for this review.
[b]Significantly different from control ($p \leq 0.05$) by Students *t*-test performed by study authors.

Source: McFarlane et al. (1997b).

Table B.39. Liver, Body Weights, and Enzyme Levels in Wistar Rat Pups Exposed to Butylated Hydroxytoluene via Gestation and Diet at Weaning (PND 21)

Parameter	Exposure Group			
	0 mg/kg-day	**25 mg/kg-day**	**100 mg/kg-day**	**250 mg/kg-day**[a]
Terminal Body weights (g)[b,]	53.8 ± 0.8	55.6 ± 0.7 (103)	54.7 ± 1.3 (102)	46.2 ± 0.9 (86)
Liver weight (g)[b]	2.24 ± 0.07	2.29 ± 0.07 (102)	2.48 ± 0.12 (111)	2.40 ± 0.13 (107)
Relative liver weight (liver/body weight %)[b,a]	4.15 ± 0.09	4.13 ± 0.10 (100)	4.51 ± 0.16 (109)	5.17 ± 0.25[c] (125)
Glucose 6-phosphatase (nmol/min/mg protein)[b]	47.7 ± 4.1	46.7 ± 3.7 (98)	39.1 ± 3.9 (82)	36.9 ± 3.4 (77)
Total glutathione (µmol/mg cytosolic protein)[b]	0.152 ± 0.012	0.128 ± 0.017 (84)	0.165 ± 0.020 (109)	0.131 ± 0.012 (86)
Glutathione *S*-transferase (µmol/min/mg protein)[b]	0.947 ± 0.102	0.999 ± 0.067 (105)	1.066 ± 0.064 (113)	2.034 ± 0.29[c] (215)
Cytochrome P-450 (nmol/mg protein)[b]	0.657 ± 0.056	0.724 ± 0.048 (110)	0.724 ± 0.053 (110)	0.812 ± 0.039 (124)
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)[b]	12.4 ± 2.2	15.3 ± 2.1 (123)	29.9 ± 7.6[c] (241)	24.1 ± 2.2[c] (194)
Benzphetamine *N*-demethylase (pmol/min/mg protein)[b]	4.58 ± 0.57	5.34 ± 0.47 (117)	9.27 ± 0.95[c] (202)	11.44 ± 1.69[c] (250)
Epoxide hydrolase (nmol/min/mg protein)[b]	3.48 ± 0.84	4.74 ± 0.91 (136)	5.67 ± 1.05 (163)	12.81 ± 2.05[c] (368)

[a]Pups of dams receiving 500 mg/kg-day.
[b]Means ± SE, (corresponding percentage); calculated for this review.
[c]Significantly different from control ($p \leq 0.05$) by Students *t*-test performed by study authors.

Source: McFarlane et al. (1997b).

Table B.40. Liver, Body Weights, and Enzyme Levels in Wistar Rat Pups Exposed to Butylated Hydroxytoluene via Gestation and Diet at 4 Weeks Postweaning (PND 49)

Parameter	Exposure Group			
	0 mg/kg-day	**25 mg/kg-day**	**100 mg/kg-day**	**250 mg/kg-day[a]**
Terminal Body weights (g)[b]	284 ± 7	294 ± 10 (104)	322 ± 6 (113)	264 ± 9 (93)
Liver weight (g)[b]	14.1 ± 0.3	14.7 ± 0.5 (104)	16.6 ± 0.4[c] (118)	14.5 ± 0.4 (103)
Relative liver weight (liver/body weight %)[b,a]	4.98 ± 0.10	5.02 ± 0.10 (100)	5.19 ± 0.15[c] (104)	5.51 ± 0.14[c] (111)
Glucose 6-phosphatase (nmol/min/mg protein)[b]	60.2 ± 6.0	61.3 ± 6.7 (102)	45.3 ± 3.4[c] (75)	43.6 ± 2.7[c] (72)
Total glutathione (µmol/mg cytosolic protein)[b]	0.130 ± 0.008	0.111 ± 0.008 (85)	0.114 ± 0.013 (87)	0.096 ± 0.007[c] (73)
Glutathione *S*-transferase (µmol/min/mg protein)[b]	1.73 ± 0.11	1.89 ± 0.10 (109)	2.48 ± 0.14[c] (143)	2.74 ± 0.13[c] (158)
Cytochrome P-450 (nmol/mg protein)[b]	0.881 ± 0.086	0.742 ± 0.032 (84)	0.740 ± 0.037 (84)	0.866 ± 0.061 (98)
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)[b]	8.91 ± 1.24	13.04 ± 1.10[c] (146)	12.31 ± 0.95[c] (138)	13.13 ± 1.65[c] (147)
Benzphetamine *N*-demethylase (pmol/min/mg protein)[b]	13.6 ± 1.4	10.8 ± 0.8 (79)	10.6 ± 1.0 (78)	14.2 ± 1.4 (96)
Epoxide hydrolase (nmol/min/mg protein)[b]	7.47 ± 1.22	8.93 ± 0.92 (120)	11.10 ± 1.00[c] (149)	14.60 ± 1.70[c] (195)

[a]Pups of dams receiving 500 mg/kg-day.
[b]Means ± SE, (corresponding percentage); calculated for this review.
[c]Significantly different from control ($p \leq 0.05$) by Students *t*-test performed by study authors.

Source: McFarlane et al. (1997b).

Table B.41. Liver, Body Weights, and Enzyme Levels in Wistar Rat Pups Exposed to Butylated Hydroxytoluene via Gestation and Diet at 22 Weeks Postweaning (PND 175)

Parameter	Exposure Group			
	0 mg/kg-day	25 mg/kg-day	100 mg/kg-day	250 mg/kg-day[a]
Terminal Body weights (g)[b,]	652 ± 21	631 ± 19 (97)	601 ± 13 (92)	570 ± 17[c] (87)
Liver weight (g)[b]	22.25 ± 0.95	21.25 ± 0.67 (96)	22.27 ± 0.55 (100)	23.73 ± 1.05 (107)
Relative liver weight (liver/body weight %)[b,a]	3.41 ± 0.10	3.39 ± 0.12 (99)	3.71 ± 0.12 (109)	4.15 ± 0.10[c] (122)
Glucose 6-phosphatase (nmol/min/mg protein)[b]	56.7 ± 3.4	54.4 ± 3.4 (96)	45.4 ± 3.3[c] (80)	43.6 ± 3.6[c] (77)
Total glutathione (µmol/mg cytosolic protein)[b]	0.123 ± 0.007	0.121 ± 0.015 (98)	0.127 ± 0.012 (103)	0.078 ± 0.008[c] (63)
Glutathione *S*-transferase (µmol/min/mg protein)[b]	1.23 ± 0.06	1.33 ± 0.05 (108)	1.45 ± 0.09 (118)	1.79 ± 0.08[c] (146)
Cytochrome P-450 (nmol/mg protein)[b]	0.567 ± 0.029	0.563 ± 0.018 (99)	0.589 ± 0.018 (104)	0.701 ± 0.025[c] (124)
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)[b]	2.45 ± 0.30	3.33 ± 0.31 (136)	3.42 ± 0.24[c] (140)	3.22 ± 0.31 (131)
Benzphetamine *N*-demethylase (pmol/min/mg protein)[b]	10.21 ± 0.70	9.62 ± 0.51 (94)	10.02 ± 0.49 (98)	11.41 ± 0.53 (112)
Epoxide hydrolase (nmol/min/mg protein)[b]	8.15 ± 1.39	11.03 ± 1.76 (135)	14.02 ± 2.70 (172)	18.26 ± 3.06[c] (224)

[a]Pups of dams receiving 500 mg/kg-day.
[b]Means ± SE, (corresponding percentage); calculated for this review.
[c]Significantly different from control ($p \leq 0.05$) by Students *t*-test performed by study authors.

Source: McFarlane et al. (1997b).

Table B.42. Summary of Data of Litters from F1 and F2 Generation Mice Administered Butylated Hydroxytoluene

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]				
	0 % (0)	**0.015% (29)**	**0.045% (88)**	**0.135% (263)**	**0.405% (790)**
F1 generation					
No. of litters	9	10	9	9	9
No. of pups	97	114	113	97	116
Litter size[a]	10.8 ± 2.28	11.4 ± 1.43 (106)	12.6 ± 2.19 (117)	10.8 ± 3.70 (100)	12.9 ± 1.54 (119)
Litter weight (g)[b]	16.33 ± 3.51	17.92 ± 2.03 (110)	19.51 ± 2.74 (119)	16.57 ± 5.25 (101)	19.49 ± 2.09 (119)
Sex ratio (male/female)	1.02 (49/48)	1.00 (57/57)	1.17 (61/52)	2.03 (65/32)	1.17 (59/57)
F2 generation					
No. of litters	10	9	9	10	10
No. of pups	117	99	106	115	109
Litter size[a]	11.7 ± 2.16	11.0 ± 2.12 (94)	11.8 ± 1.92 (101)	11.5 ± 2.68 (98)	10.9 ± 2.56 (93)
Litter weight (g)[b]	18.03 ± 2.94	17.50 ± 3.06 (17.5)	17.72 ± 1.78 (17.72)	17.67 ± 3.05 (17.67)	16.92 ± 3.41 (16.92)
Sex ratio (male/female)	1.09 (61/56)	1.02 (50/49)	1.16 (57/49)	1.35 (66/49)	0.70 (45/64)

[a]Doses were converted from % of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), and then ppm intake in food is adjusted using
[b]Each value represents the mean ± SD, (corresponding percentage of controls is shown in parentheses); calculated for this review.
the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).

Source: Tanaka et al. (1993).

Table B.43. Selected Behavioral Observations from F1 Generation Mice Administered Butylated Hydroxytoluene

Parameter	Postnatal Day (PND)	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]				
		0% (0)	0.015% (29)	0.045% (88)	0.135% (263)	0.405% (790)
Surface Righting[b]	7	1.47 ± 0.325	1.84 ± 0.139[c] (125)	1.69 ± 0.248 (115)	1.43 ± 0.400 (97)	1.64 ± 0.274 (112)
Ambulation (males)[b,d]	21	93.1 ± 37.68	100.4 ± 47.66 (108)	49.6 ± 21.70[c] (53)	79.1 ± 66.01 (85)	71.3 ± 48.14 (77)
Ambulation (females)[b,e]	21	120.3 ± 53.42	145.8 ± 29.48 (121)	101.8 ± 52.42 (85)	125.7 ± 68.03 (104)	111.2 ± 38.80 (92)
180° turn (males)[b,d]	21	5.0 ± 1.85	5.7 ± 2.36 (114)	3.8 ± 2.49 (76)	3.8 ± 1.64 (76)	4.3 ± 2.24 (86)
180° turn (females)[b,e]	21	5.6 ± 2.20	6.0 ± 1.41 (107)	4.8 ± 2.38 (86)	5.7 ± 1.94 (102)	4.3 ± 1.00 (77)

[a]Doses were converted from % of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), and then ppm intake in food is adjusted using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Each value represents the mean ± SD, (corresponding percentage of control); calculated for this review.
[c]Significantly different from controls ($p < 0.05$) by unspecified methods.
[d]Different numbers of males were observed (8, 10, 8, 9, and 9; respectively).
[e]Different numbers of females were observed (8, 10, 8, 9, and 9; respectively).

Source: Tanaka et al. (1993).

Table B.44. Selected Behavioral Observations from F2 Generation Mice Administered Butylated Hydroxytoluene

Parameter	Postnatal Day (PND)	Exposure Group (Adjusted Daily Dose, mg/kg-day)[a]				
		0 % (0)	0.015% (29)	0.045% (88)	0.135% (263)	0.405% (790)
Surface Righting[b]	4	0.24 ± 0.137	0.50 ± 0.261 (208)	0.26 ± 0.264 (108)	0.48 ± 0.370[c] (200)	0.45 ± 0.593 (188)
Negative Geotaxis[b]	4	1.32 ± 0.286	1.30 ± 0.304 (98)	1.42 ± 0.412 (108)	1.45 ± 0.368 (110)	1.68 ± 0.422[c] (127)
Ambulation (males)[b,d]	21	193.1 ± 88.43	121.7 ± 61.34 (63)	141.8 ± 25.17[c] (73)	149.3 ± 66.75 (77)	150.1 ± 39.47 (78)
Ambulation (females)[b,e]	21	173.0 ± 67.87	160.2 ± 42.61 (93)	97.2 ± 36.05[f] (56)	177.9 ± 46.72 (103)	161.8 ± 52.58 (94)
180° turn (males)[b,d]	21	9.2 ± 3.08	4.6 ± 2.35[f] (50)	5.3 ± 2.55[c] (58)	4.8 ± 2.86[f] (52)	4.1 ± 2.28[f] (45)
180° turn (females)[b,e]	21	6.8 ± 4.32	6.4 ± 4.42 (94)	3.4 ± 2.30 (50)	6.6 ± 3.57 (97)	5.3 ± 1.49 (78)

[a]Doses were converted from % of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), and then ppm intake in food is adjusted using the following equation:
$Dose_{ADJ}$ = Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days).
[b]Each value represents the mean ± SD, () –corresponding percentage calculated for this review.
[c]Significantly different from controls ($p < 0.05$) by the Mann-Whitney U-test conducted by the study authors.
[d]Different numbers of males were observed (10, 9, 9, 10, and 10; respectively).
[e]Different numbers of females were observed (10, 9, 9, 10, and 10; respectively).
[f]Significantly different from controls ($p < 0.01$) by the Mann-Whitney U-test conducted by the study authors.

Source: Tanaka et al. (1993).

Table B.45. Behavioral Changes in F1 Swiss-Webster Mice Exposed to 0.5% BHT from Gestation Through PND 42

Parameter	Measurement	Exposure Group (Adjusted Daily Dose, mg/kg-day)	
		Control	BHT
Mouse City Behaviors	Exploration	7.95 ± 2.36	7.87 ± 3.11
	Sleeping	0.58 ± 1.57	0.18 ± 0.70[a]
	Grooming Self	1.05 ± 1.05	0.95 ± 1.31
Observation/Mouse Session	Fighting	0.97 ± 2.15	4.75 ± 8.52[a]
Climbing Screen	Mean base time (sec) first 5 trials	5.86 ± 0.21	5.42 ± 0.61
	Mean base time (sec) second 5 trials	4.72 ± 0.23[b]	5.40 ± 0.25
	Mean climbing time (sec) per trial	2.75 ± 1.84	2.86 ± 0.32
Isolation-Induced Aggression	% Fighters	31	62
	Mean rank of latency to onset of aggression	53.9	35.1[b]
Activity	Orientation reflex in counts per hr per mouse	2744	2440
	Psychomotor activity in counts per hr per mouse	575	601

[a]Statistically significant compared to control ($p < 0.05$) by the Wilcoxon 2-sample rank test conducted by the study authors.
[b]Statistically significant compared to control ($p < 0.005$) by the Wilcoxon 2-sample rank test conducted by the study authors.

Source: Stokes and Scudder (1974).

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE CHRONIC p-RfD AND p-OSF

BENCHMARK DOSE CALCULATIONS FOR THE CHRONIC p-RfD

Modeling Procedure for Dichotomous Data

The BMD modeling of dichotomous data was conducted with EPA's BMDS (version 2.1.2). For these data, all of the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-probit, Weibull, and Quantal-linear models) available within the software were fit using a BMR of 10% extra risk. Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD

Modeling Procedure for Continuous Data

The BMD modeling of continuous data was conducted with EPA's BMDS (version 2.1.2). For these data (e.g., increased relative liver weight), all continuous models available within the software were fit using a BMR of 10% relative risk. An adequate fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging 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 a 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*-value < 0.1), the dataset was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive the RfD.

Focal Alveolar Histiocytosis in Rats Treated with BHT in The Diet For 105 Weeks (NCI, 1979c)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of alveolar histiocytosis in F344 female rats fed BHT in the diet for 105 weeks (NCI, 1979c; Table B.18) For the female rat data, all dose groups were included in the analysis (see Table C.1 and Figure C.1). As assessed by the χ^2 goodness-of-fit statistic, the Logistic, Probit, Log-probit, and Quantal-linear models adequately fit the data. The Probit model provided the best fit, as assessed by AIC, for data from female rats (see Table C.1 and Figure C.1). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 199 and 157 mg/kg-day in female rats.

Table C.1. BMD Dose Response Modeling Results Based on the Incidence of Focal Alveolar Histiocytosis in Female F344 Rats Fed BHT in the Diet for 105 Weeks.

Model	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	N/A	139.467	197	91
Multistage[b]	N/A	139.467	188	91
Logistic	0.8707	137.494	210	166
Log-logistic[c]	N/A	139.464	199	75
Probit	**0.9295**	**137.475**	**199**	**157**
Log-probit[c]	0.9022	137.482	219	155
Weibull[a]	N/A	139.467	194	91

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.

N/A = Not Applicable

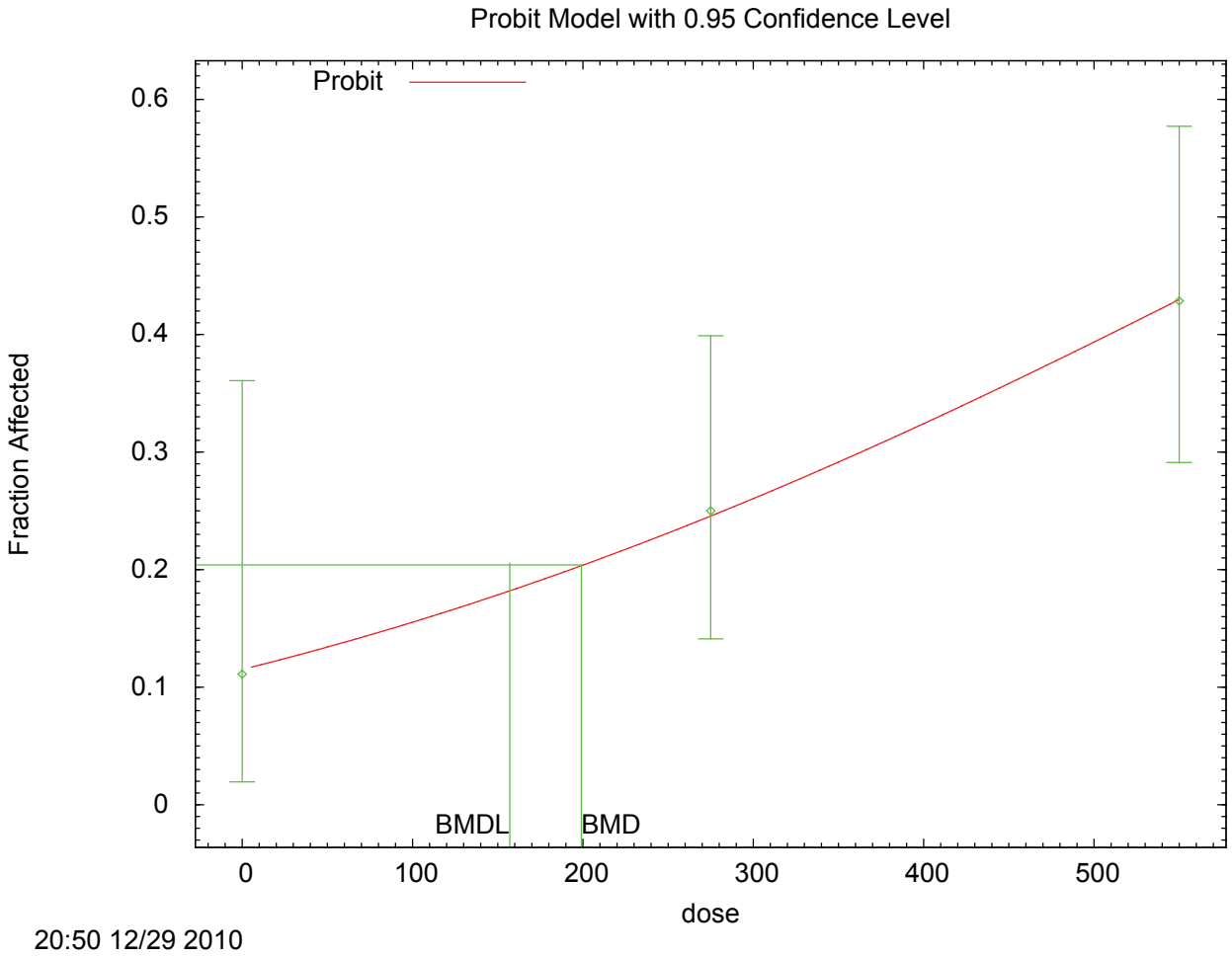

Figure C.1. Dose Response Modeling of Incidence Data for Focal Alveolar Histiocytosis in Female F344 Rats Fed BHT in the Diet for 105 Weeks.

Hepatocytomegaly in Mice Treated with BHT in the Diet for 107 Weeks (NCI, 1979d)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of hepatocytomegaly in male $B6C3F_1$ mice fed BHT in the diet for 107 weeks (NCI, 1979d; Table B.30) For these data, all dose groups were included in the analysis (see Table C.2 and Figure C.2). As assessed by the χ^2 goodness-of-fit statistic, all available models adequately fit the data. The Log-probit model provided the best fit, as assessed by AIC, for data from male mice (see Table C.2 and Figure C.2). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 355 and 284 mg/kg-day in male mice.

Table C.2. BMD Dose Response Modeling Results Based on the Incidence of Hepatocytomegaly in Male $B6C3F_1$ Mice Fed BHT in the Diet for 107 Weeks.

Model	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	1	116.593	316	170
Multistage[b]	0.9999	116.593	295	170
Logistic	0.2392	118.79	471	375
Log-logistic[c]	1	116.593	324	143
Probit	0.2855	118.344	445	353
Log-probit[c]	**0.9909**	**114.612**	**355**	**284**
Weibull[a]	1	116.593	310	170

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.

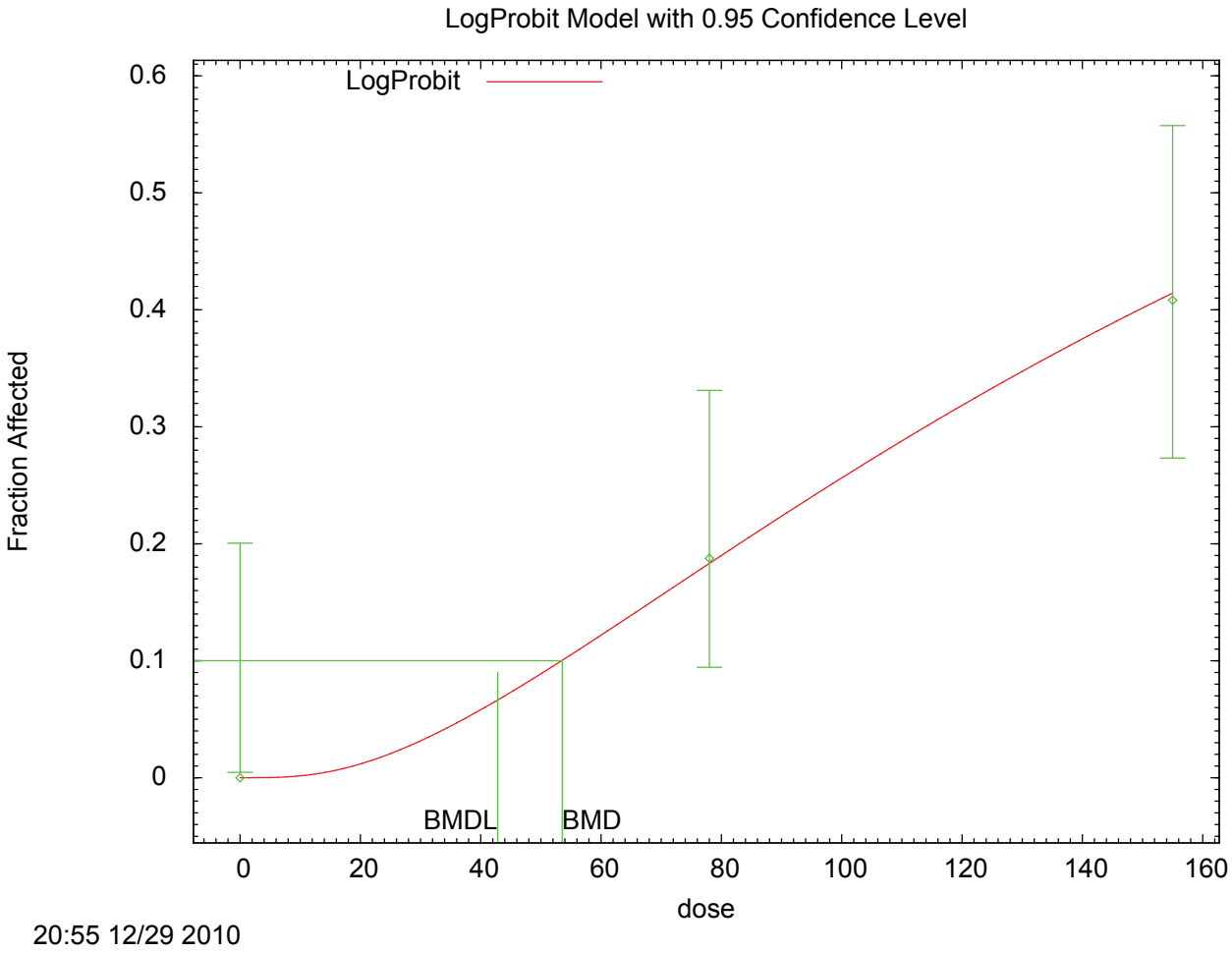

Figure C.2. Dose Response Modeling of Incidence Data for Hepatocytomegaly in Male B6C3F$_1$ Mice Fed BHT in the Diet for 107 Weeks.

Liver Peliosis in Mice Treated with BHT in the Diet for 107 Weeks (NCI, 1979d)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of liver peliosis in male $B6C3F_1$ mice fed BHT in the diet for 107 weeks (NCI, 1979d; Table B.30) For these data, all dose groups were included in the analysis (see Table C.3 and Figure C.3). As assessed by the χ^2 goodness-of-fit statistic, all available models besides Logistic and Log-probit adequately fit the data. The Log-Logistic model was considered the best fit and produced a BMD_{10} and $BMDL_{10}$ of 73 and 14 mg/kg-day.

Table C.3. BMD Dose Response Modeling Results Based on the Incidence of Liver Peliosis in Male $B6C3F_1$ Mice Fed BHT in the Diet for 107 Weeks.

Model	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	0.8353	96.119	47	38
Multistage[b]	0.7527	89.744	45	36
Logistic	0.0047	108.056	137	103
Log-logistic[c]	**1**	**97.765**	**73**	**14**
Probit	0.0034	108.901	133	103
Log-probit[c]	0.9727	95.82	85	66
Weibull[a]	0.8353	96.119	47	38

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.

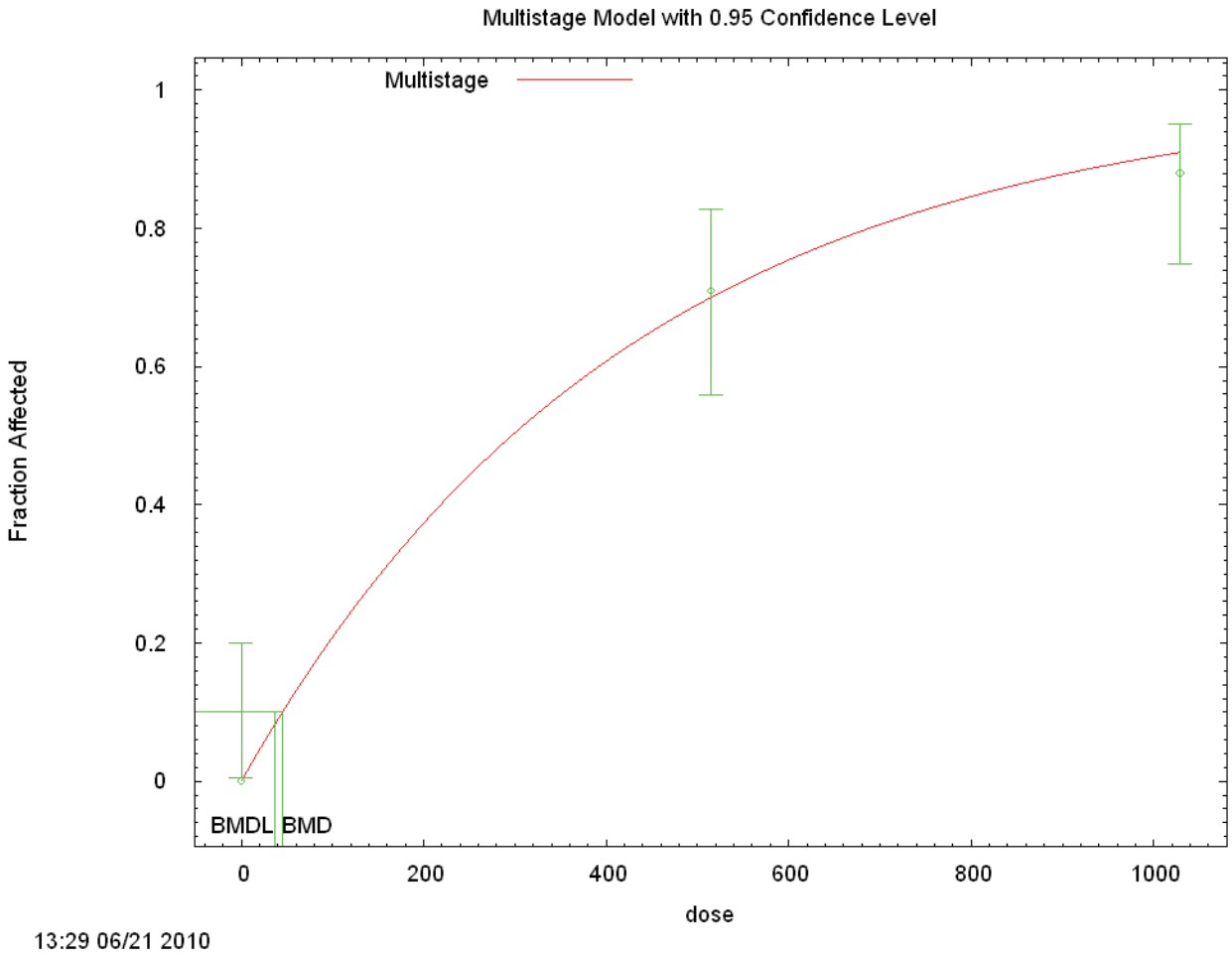

Figure C.3. Multistage Model for Male Liver Peliosis Data (NCI, 1979d)

Liver Necrosis in Mice Treated with BHT in the Diet for 107 Weeks (NCI, 1979d)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of liver necrosis in male B6C3F$_1$ mice fed BHT in the diet for 107 weeks (NCI, 1979d; Table B.30). For these data, all dose groups were included in the analysis (see Table C.4 and Figure C.4). As assessed by the χ^2 goodness-of-fit statistic, the log-logistics models adequately fit the data. The Quantal-linear model provided the best fit, as assessed by AIC, for data from male mice (see Table C.4 and Figure C.4). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 142 and 16 mg/kg-day.

Table C.4. BMD Dose Response Modeling Results Based on the Incidence of Liver Necrosis in Male B6C3F$_1$ Mice Fed BHT in the Diet for 107 Weeks.

Model	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	N/A	104.129	65	37
Multistage[b]	N/A	96.88	52	34
Logistic	0.1565	104.149	117	88
Log-logistic[c]	**N/A**	**104.129**	**142**	**16**
Probit	0.1022	104.879	113	90
Log-probit[c]	N/A	104.129	132	65
Weibull[a]	N/A	104.129	59	37

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.

N/A = Not Applicable

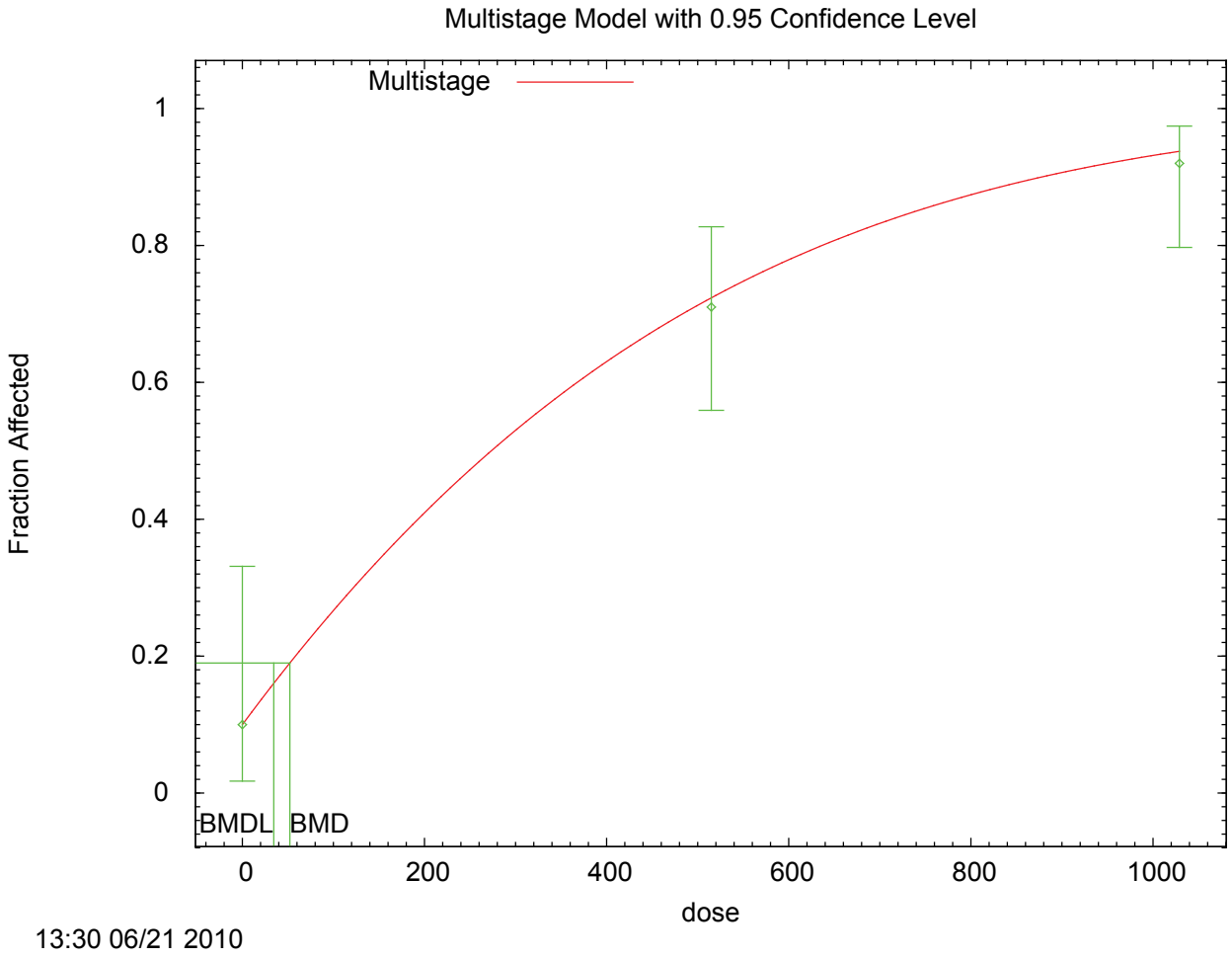

Figure C.4. Dose Response Modeling of Incidence Data for Liver Necrosis in Male B6C3F$_1$ Mice Fed BHT in the Diet for 107 Weeks.

Hepatic Cytoplasmic Vacuolation in Mice Treated with BHT in the Diet for 107 Weeks (NCI, 1979d)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of hepatic cytoplasmic vacuolation in male $B6C3F_1$ mice fed BHT in the diet for 107 weeks (NCI, 1979d; Table B.30) For these data, all dose groups were included in the analysis (see Table C.5 and Figure C.5). As assessed by the χ^2 goodness-of-fit statistic, all available models adequately fit the data. The Log-logistic model provided the best fit, as assessed by AIC, for data from male mice (see Table C.5 and Figure C.5). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 182 and 102 mg/kg-day.

Table C.5. BMD Dose Response Modeling Results Based on the Incidence of Hepatic Cytoplasmic Vacuolation in Male $B6C3F_1$ Mice Fed BHT in the Diet for 107 Weeks.

Model	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	0.2471	154.989	232	142
Multistage[b]	0.2464	154.797	234	143
Logistic	0.1489	155.794	356	256
Log-logistic[c]	**0.3377**	**154.567**	**182**	**102**
Probit	0.1547	155.726	344	246
Log-probit[c]	0.1040	156.301	400	252
Weibull[a]	0.2471	154.989	232	142

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.

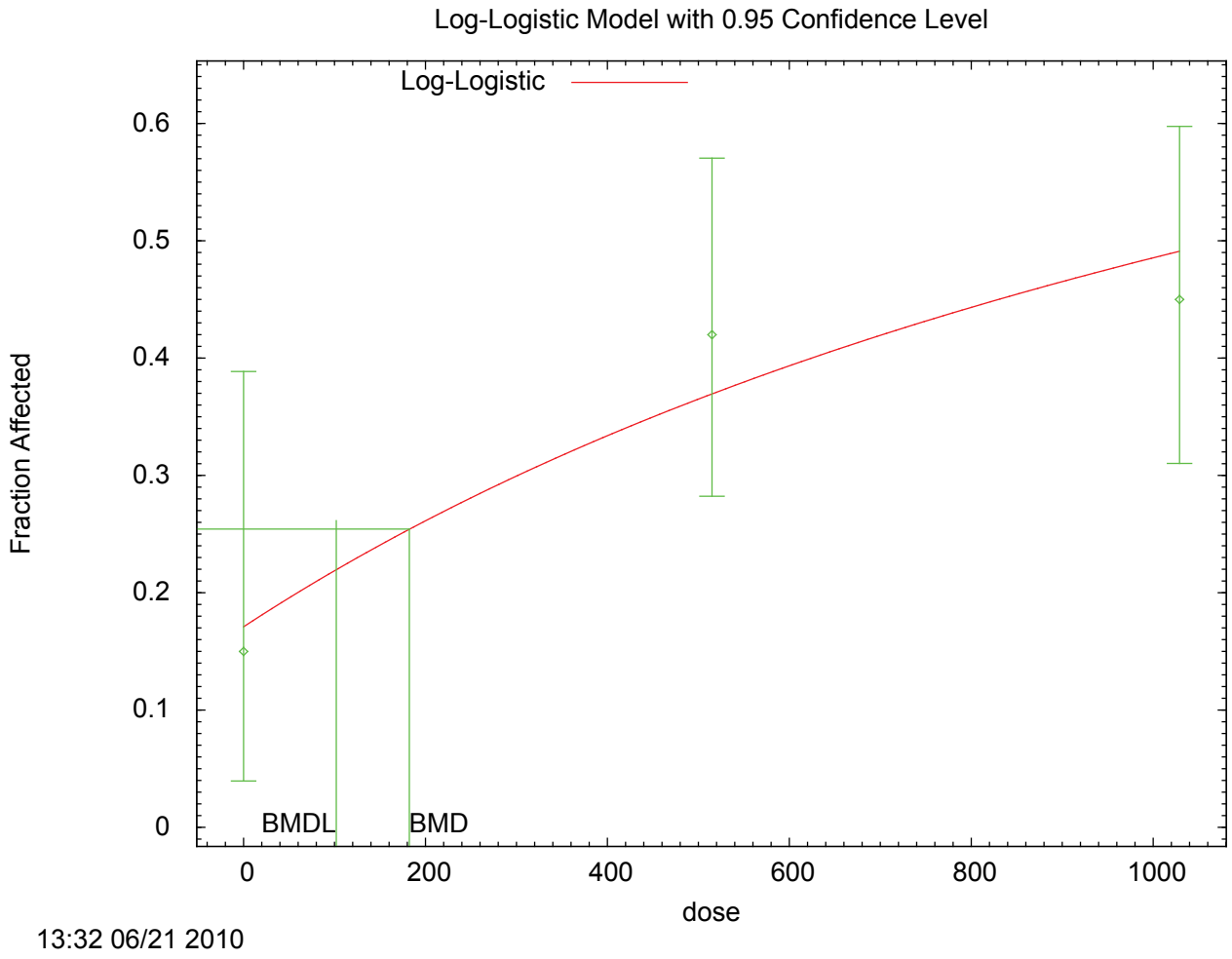

Figure C.5. Dose Response Modeling of Incidence Data for Hepatic Cytoplasmic Vacuolation in Male B6C3F1 Mice Fed BHT in the Diet for 107 Weeks.

Decreased Terminal Body Weight of F0 Wistar Rat Dams treated with BHT in the Diet for 14 Weeks (McFarlane et al., 1997a)

All available continuous models in BMDS (version 2.1.2) were fit to the decreased terminal body weight data from Wistar rat dams exposed to BHT in the diet prior to and during mating and throughout gestation and lactation (for a total of 14 weeks) (McFarlane et al., 1997a; Table B.34). The Power model in BMDS provided an adequate fit to the data, and Test 2 (p = 0.2918) also indicated that using a constant variance model was appropriate for modeling these data. Thus, all of the BMD modeling results shown in Table C.6 and Figure C.6 were obtained from constant variance models. Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 730 and 664 mg/kg-day.

Table C.6. BMD Modeling Results on Decreased Terminal Body Weight in Rat Dams Exposed to BHT for 14 Weeks

Model	Test 2	Test 3	χ^2 p-Value	AIC	BMD_{10}	$BMDL_{10}$
Linear	0.2918	0.2918	<.0001	190.275	516	457
Polynomial	0.2918	0.2918	<.0001	240.509	-9999	2123
Power	**0.2918**	**0.2918**	**0.8433**	**168.491**	**730**	**664**
Hill	0.2918	0.2918	N/A	170.498	729	664

N/A = Not Applicable

Figure C.6. Dose Response Modeling of Decreased Body Weight Data in Wistar Rat Dams Fed BHT in the Diet for 14 Weeks.

Increased Relative Liver Weight of Wistar Rat Dams Treated with BHT in the Diet for 14 Weeks (McFarlane et al., 1997a)

All available continuous models in BMDS (version 2.1.2) were fit to the increased relative liver weight data from Wistar rat dams exposed to BHT in the diet prior to and during mating and throughout gestation and lactation (for a total of 14 weeks) (McFarlane et al., 1997a; Table B.34). No available model in BMDS provided an adequate fit to the data as Test 4 for all models was less than 0.1. All of the BMD modeling results shown in Table C.7 were obtained from constant variance models. Because all models for these data failed, a BMD output graph is not provided.

Table C.7. BMD Modeling Results on Increased Relative Liver Weight in Rat Dams Exposed to BHT for 14 Weeks

Model	Test 2	Test 3	χ^2 *p*-Value	AIC	BMD_{10}	$BMDL_{10}$
Linear	0.8389	0.7996	<.0001	-5.134	54	32
Polynomial	0.8389	0.7996	<.0001	-5.134	54	32
Power	0.8389	0.7996	<.0001	-5.134	54	32
Hill	0.8389	0.7996	<.0001	-21.464	7.8×10^{-13}	7.8×10^{-13}

Decreased Body Weight of Wistar Rat Pups Treated with BHT in the Diet for 3 Weeks (McFarlane et al., 1997a)

All available continuous models in BMDS (version 2.1.2) were fit to the decreased body-weight data from Wistar rat pups exposed to BHT in the diet throughout gestation and lactation (for a total of 3 weeks) (McFarlane et al., 1997a; Table B.35). No available model in BMDS provided an adequate fit to the data as Test 4 for all models was less than 0.1. All of the BMD modeling results shown in Table C.8 were obtained from constant variance models. Because all models for these data failed, a BMD output graph is not provided.

Table C.8. BMD Modeling Results on Decreased Body Weight in Rat Pups Exposed to BHT for 3 Weeks

Model	Test 2	Test 3	χ^2 *p*-Value	AIC	BMD_{10}	$BMDL_{10}$
Linear	<.0001	<.0001	<.0001	213.523	150	145
Polynomial	<.0001	<.0001	<.0001	78.479	150	145
Power	<.0001	<.0001	<.0001	213.523	150	145
Hill	<.0001	<.0001	<.0001	213.523	150	0.000499

APPENDIX D. BENCHMARK DOSE CALCULATIONS FOR THE ORAL SLOPE FACTOR

Model-Fitting Procedure for Cancer Incidence Data

The model-fitting procedure for dichotomous cancer incidence data is as follows. The Multistage-cancer model in the EPA benchmark dose software (BMDS) 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: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all the models providing adequate fit to the data, the lowest bound of the BMD (BMDL) is selected as the point of departure when the difference between the BMDLs estimated from these models is more than three-fold (unless it appears to be an outlier); otherwise, the BMDL from the model with the lowest (Akaike Information Criterion) AIC is chosen. In accordance with EPA (2000) guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated.

Model-Fitting Results for Hepatocellular Adenomas and Total Hepatocellular Tumors in Wistar F1 Rats (Olsen et al., 1986)

Table B.25 shows the dose-response data on hepatocellular tumors in Wistar rats administered BHT via diet for 141−144 weeks (Olsen et al., 1986). Modeling was performed according to the procedure outlined above using BMDS version 2.1.2 with parameter restrictions for rats based on the duration-adjusted HEDs shown in Table 2. Model predictions are shown in Table 13. For incidence of hepatocellular adenomas in both male and female rats, the multistage-cancer model provided an adequate fit (goodness-of-fit p-value > 0.1). The 2-degree polynomial model yielded a BMD_{10HED} value of 42 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10HED}$) of 30 mg/kg-day for male rats. The 1-degree polynomial model yielded a BMD_{10HED} value of 58 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10HED}$) of 36 mg/kg-day for female rats. The fit of the multistage-cancer models to the hepatocellular adenoma incidence data for male and female rats is shown in Table 14 and Figures D.1-D.4. For incidence of total hepatocellular tumors in both male and female rats, the 2-degree polynomial model yielded a BMD_{10HED} value of 41 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10HED}$) of 28 mg/kg-day for male rats. The 1-degree polynomial model yielded a BMD_{10HED} value of 49 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10HED}$) of 32 mg/kg-day for female rats.

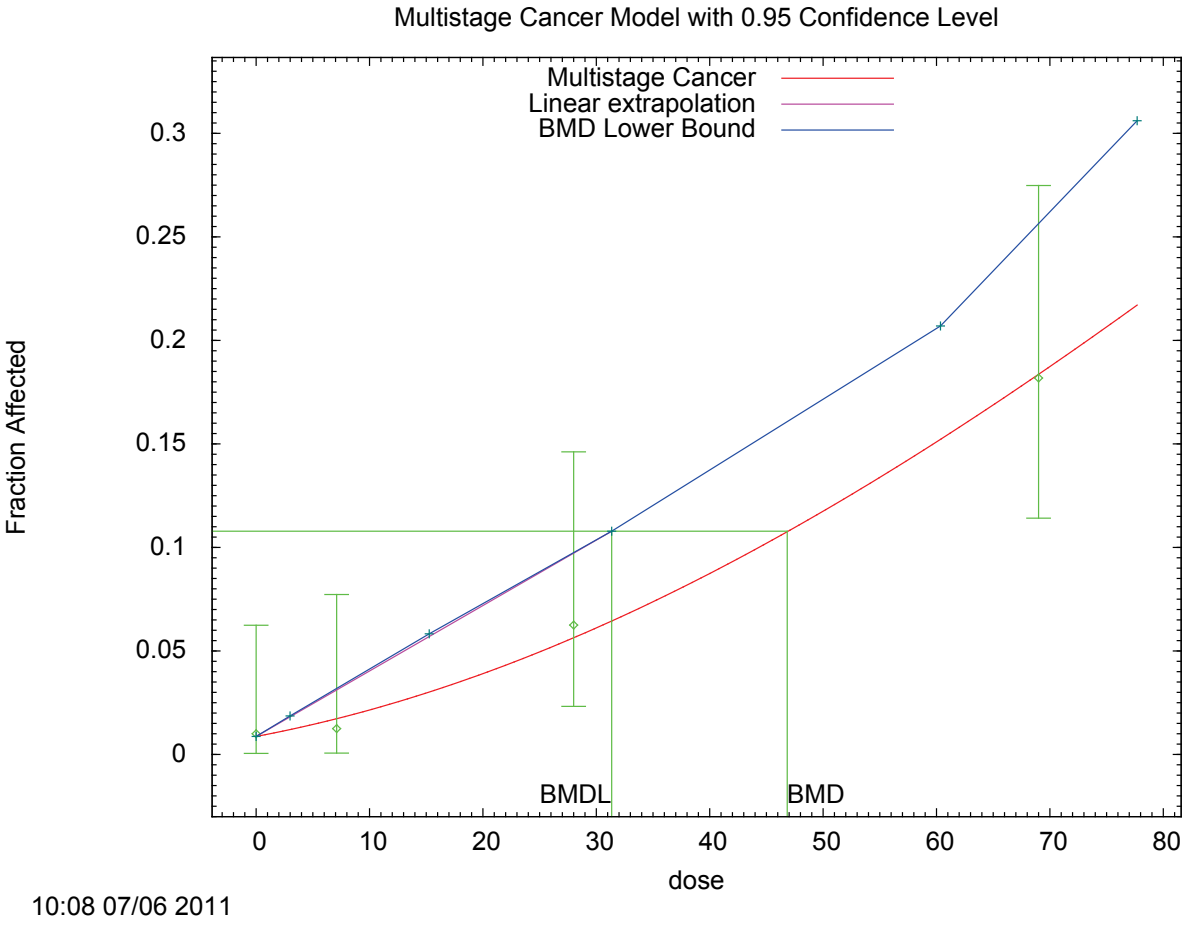

Figure D.1. Multistage Cancer Model for Male Liver Adenoma Data (Olsen et al. [1986])

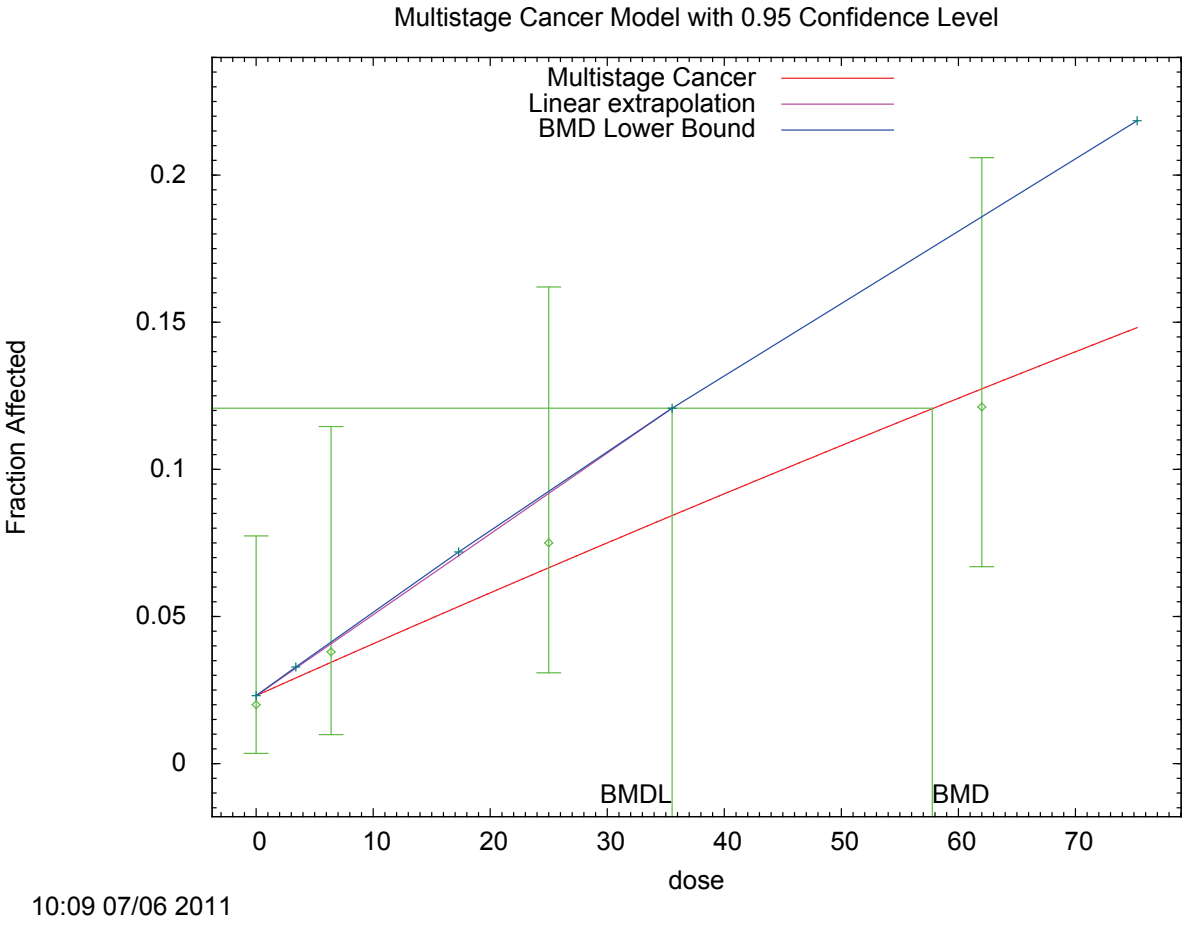

Figure D.2. Multistage Cancer Model for Female Liver Adenoma Data (Olsen et al., 1986)

```
  Dependent variable = Response
  Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =     0.013896
                        Beta(1) =  0.000749719
                        Beta(2) = 5.02883e-005


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)      Beta(2)

Background            1        -0.62         0.48

   Beta(1)        -0.62            1        -0.97

   Beta(2)         0.48        -0.97            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0165429            *               *                   *
        Beta(1)      0.000143576            *               *                   *
        Beta(2)     5.96679e-005            *               *                   *

* - Indicates that this value is not calculated.


                       Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -93.4932         4
   Fitted model          -93.74         3      0.493526      1          0.4824
  Reduced model        -114.715         1       42.4428      3         <.0001

           AIC:          193.48


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size          Residual
  ---------------------------------------------------------------------------
```

```
    0.0000     0.0165         1.654     2.000         100        0.271
    7.1000     0.0205         1.640     1.000          80       -0.505
   28.0000     0.0653         5.220     6.000          80        0.353
   69.0000     0.2670        26.437    26.000          99       -0.099

 Chi^2 = 0.46      d.f. = 1        P-value = 0.4964


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        40.8353

            BMDL =        28.2683

            BMDU =         49.528

Taken together, (28.2683, 49.528 ) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00353753
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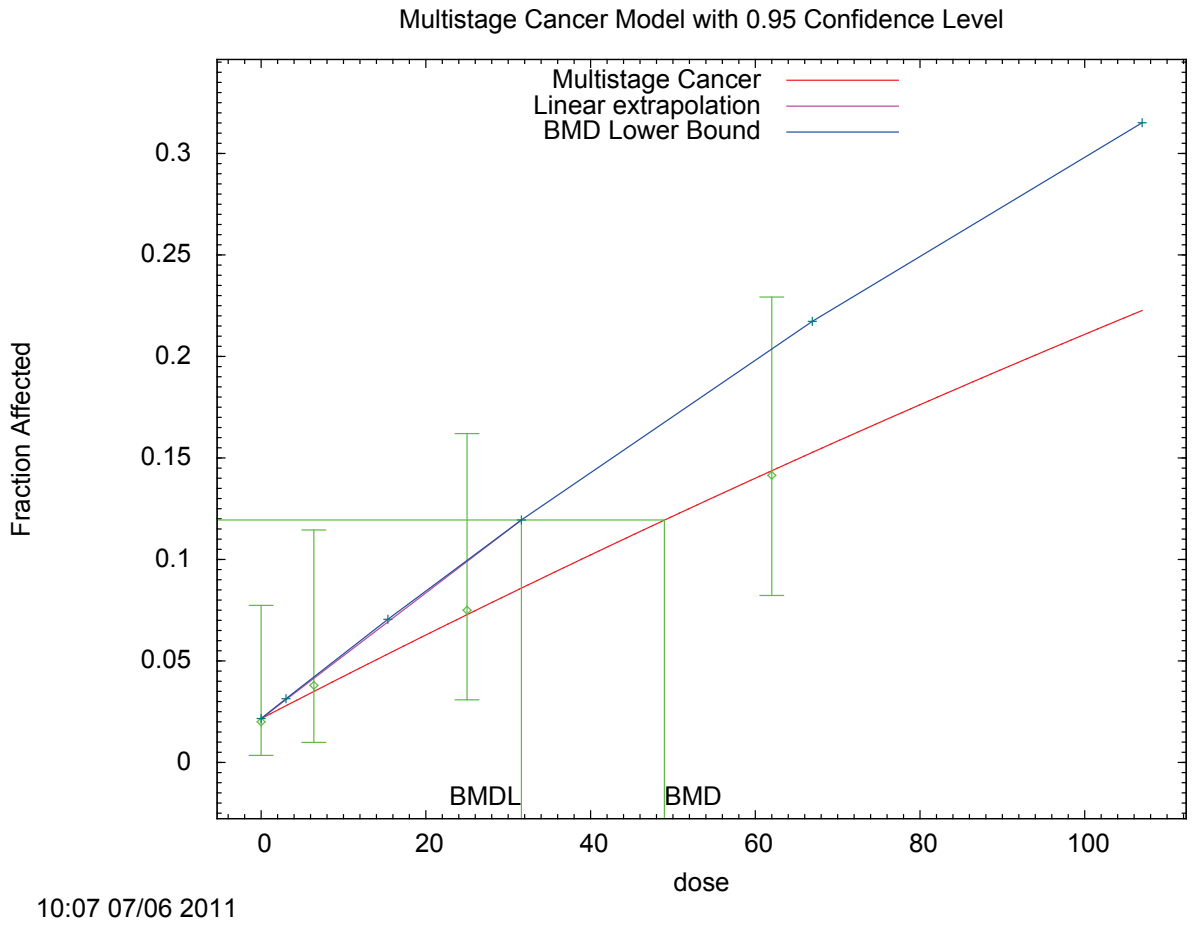

Figure D.4. Multistage Cancer Model for Female Total Hepatocellular Tumor Data (Olsen et al., 1986)

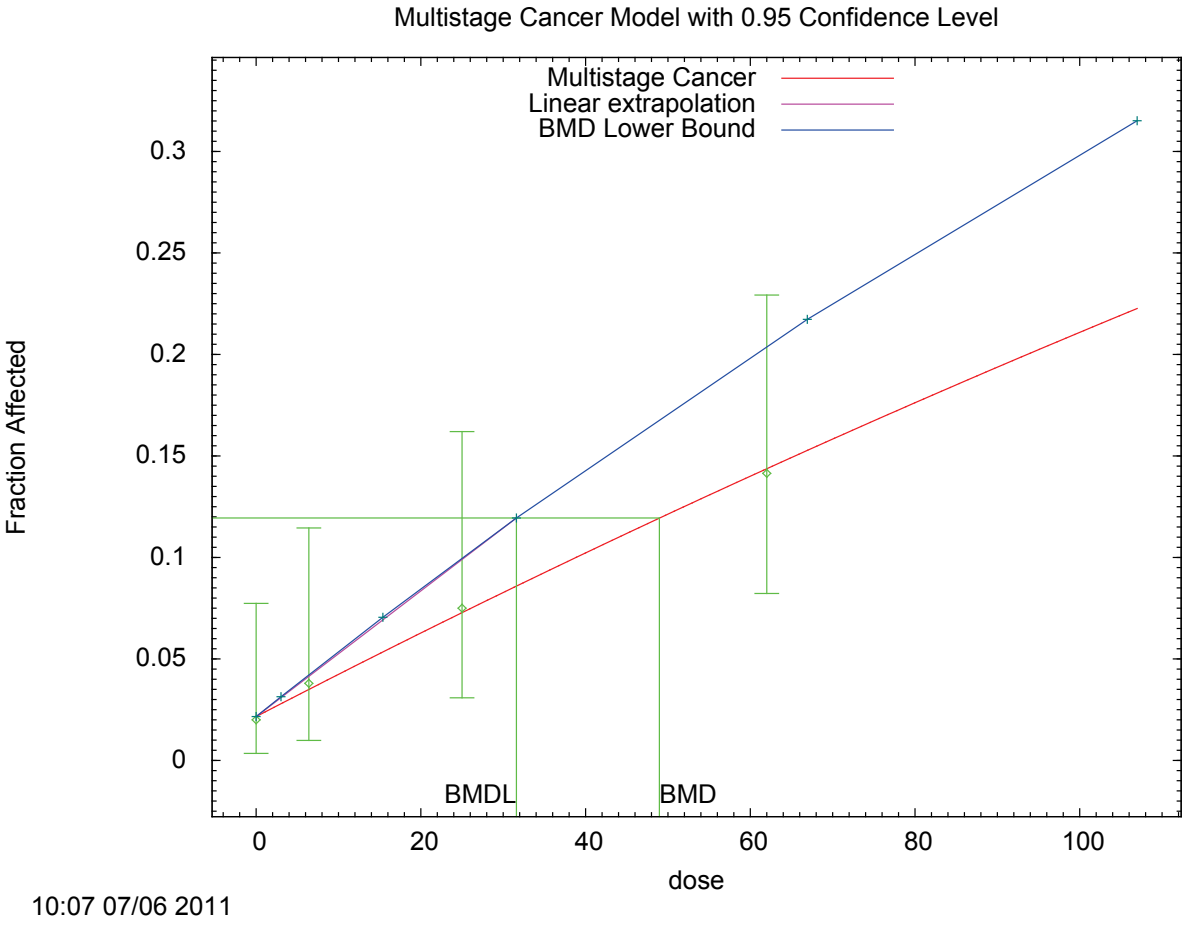

Figure D.4. Multistage Cancer Model for Female Total Hepatocellular Tumor Data (Olsen et al., 1986)

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