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TOXICOLOGICAL REVIEW

OF

BIPHENYL

(CAS No. 92-52-4)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2011

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LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's Information Criterion
ALT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
BBN	N-butyl-N-(4-hydroxybutyl)nitrosamine
BMD	benchmark dose
BMDL	95% lower confidence limit on the BMD
BMR	benchmark response
BMDS	Benchmark Dose Software
BrdU	5-bromo-2-deoxyuridine
BUN	blood urea nitrogen
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CVSF	conduction velocity of the slowest motor fibers
CYP	cytochrome P-450
DNA	deoxyribonucleic acid
EEG	electroencephalography
EHEN	N-ethyl-N-hydroxyethylnitrosamine
EMG	electromyographic
ENMG	electroneuromyography
GC	gas chromatography
GD	gestation day
GOT	glutamate oxaloacetate transaminase
GPT	glutamate pyruvate transaminase
HED	human equivalent doses
HGPRT	hypoxanthine guanine phosphoribosyl transferase
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal or intraperitoneally
IRIS	Integrated Risk Information System
K_{ow}	octanol/water partition coefficient
K_m	Michaelis constant
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
MCV	motor conduction velocity
MS	mass spectrometry
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PD	Parkinson's disease
POD	point of departure

PPAR	peroxisome proliferator activated receptors
RD	relative deviation
RfC	reference concentration
RfD	reference dose
ROS	reactive oxygen species
RR	relative risk
SCE	sister chromatid exchange
SD	standard deviation
SULT	sulphotransferase
TLV	threshold limit value
TMS	trimethylsilyl
TWA	time-weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factors
UGT	uridine diphosphate glucuronosyl transferase
U.S. EPA	U.S. Environmental Protection Agency

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to biphenyl. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of biphenyl.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of biphenyl. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a plausible inhalation unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for biphenyl has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986a), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies* (U.S. EPA, 1994a), *Methods for*

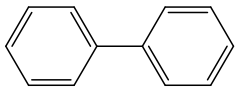
Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000a), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006b), *A Framework for Assessing Health Risk of Environmental Exposures to Children* (U.S. EPA, 2006a), and *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011).

The literature search strategy employed for biphenyl was based on the chemical name, Chemical Abstracts Service Registry Number (CASRN), and multiple common synonyms. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. Primary, peer-reviewed literature identified through August 2011 was included where that literature was determined to be critical to the assessment. The relevant literature included publications on biphenyl that were identified through Toxicology Literature Online (TOXLINE), PubMed, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate.

2. CHEMICAL AND PHYSICAL INFORMATION

Pure biphenyl is a white or colorless crystalline solid that usually forms leaflets or scales; commercial preparations may be yellowish or slightly tan (HSDB, 2005). Biphenyl is said to have a pleasant odor that is variably described as peculiar, butter-like, or resembling geraniums (HSDB, 2005; Boehncke et al., 1999). Biphenyl melts at 69°C and has a vapor pressure of 8.93×10^{-3} mm Hg at 25°C, making it likely to enter the environment in its vaporized form (HSDB, 2005). If particle-bound biphenyl is precipitated to the ground, it is likely to be reintroduced to the atmosphere by volatilization. The water solubility of biphenyl is 7.48 mg/L at 25°C. The logarithm of the octanol/water partition coefficient (K_{ow}) of biphenyl of 3.98 suggests a potential for bioaccumulation (HSDB, 2005). Because it is biodegraded with an estimated half-life of 2 and 3 days in air and water, respectively (HSDB, 2005), and is metabolized rapidly by humans and animals (see Section 3), bioaccumulation does not occur (Boehncke et al., 1999). Biphenyl is ubiquitous in the environment, with reported indoor air concentrations of 0.16–1 µg/m^3 and outdoor levels of approximately 0.03 µg/m^3 (Boehncke et al., 1999). The physicochemical properties of biphenyl are summarized in Table 2-1.

Table 2-1. Physicochemical properties of biphenyl

Synonyms	Diphenyl, 1,1′-biphenyl, 1,1′-diphenyl, bibenzene, phenylbenzene, lemonene, Carolid AL, Phenador-X, Tetrosine LY
CASRN	92-52-4
Chemical structure	
Chemical formula	$C_{12}H_{10}$
Molecular weight	154.2
Melting point	69°C
Boiling point	256°C
Specific gravity	1.041 g/cm^3 at 20°C
Vapor pressure	8.93×10^{-3} mm Hg at 25°C
Log K_{ow}	4.01; 4.11[a]; 4.17 or 5.27–5.46[b]
Water solubility	7.48 mg/L at 25°C
Henry's law constant	3.08×10^{-4} atm-m^3/mol at 25°C
Conversion factors	1 ppm = 6.31 mg/m^3; 1 mg/m^3 = 0.159 ppm

[a]Monsanto (1946).
[b]Estimated by different methods: Dow Chemical Co. (1983).

Source: HSDB (2005).

Biphenyl exists naturally as a component of crude oil or coal tar. It is primarily produced by debromination/dimerization of bromobenzene, is isolated as a byproduct of the hydrodealkylation of toluene (yield approximately 1%), or is synthesized by catalytic dehydrocondensation of benzene. Biphenyl is currently not registered for use as a pesticide in the United States, but is still used in other countries as a fungistat, most commonly to preserve packaged citrus fruits or in plant disease control (HSDB, 2005). The current major uses of biphenyl are as chemical synthesis intermediates (among them, the sodium salt of 2-hydroxy-biphenyl, a pesticide known as Dowicide 1), as dye carriers in polyester dyeing, and as components in heat transfer fluids (in particular Dowtherm A or Therminol® VP-1, consisting of 26.5% biphenyl and 73.5% diphenyl oxide). Historically, biphenyl was the primary byproduct in the manufacture of polychlorinated biphenyls (PCBs) until PCBs were banned in the 1970s (U.S. EPA, 1978). The purity of technical biphenyl ranges from 93 to 99.9%. The prevalent impurities in technical preparations are terphenyls, a side product from the dehydrocondensation of benzene. Biphenyl is rated as a high-volume production chemical. Annual U.S. production in 1990 was approximately 1.6×10^4 metric tons (HSDB, 2005).

3. TOXICOKINETICS

3.1. ABSORPTION

No quantitative studies on the absorption of biphenyl have been conducted in humans. Animal studies in rats, rabbits, guinea pigs, and pigs indicate that biphenyl is rapidly and readily absorbed following oral exposure, as evidenced by the detection of metabolites in urine and bile (Meyer, 1977; Meyer and Scheline, 1976; Meyer et al., 1976a; Meyer et al., 1976b). Results from a study with rats administered radiolabeled biphenyl indicate extensive oral absorption (Meyer et al., 1976b) (see below), whereas results from studies of rabbits, guinea pigs, and pigs administered nonlabeled biphenyl indicate less extensive oral absorption in the range of 28–49% of the administered dose (Meyer, 1977; Meyer et al., 1976a).

In the most quantitative assessment of absorption using radiolabeled biphenyl, male albino rats (n = 3; body weight = 200–300 g) given an oral dose of 100 mg/kg (0.7–1.0 μCi) of [^{14}C]-biphenyl (in soy oil) excreted 75–80% of the radioactivity in their urine within the first 24 hours, with a total average urinary excretion of 84.8% and fecal excretion of 7.3% during the 96-hour postdosing period (Meyer et al., 1976b). Only a trace of [^{14}C]-CO_2 was detected in expired air and <1% of the radioactivity was recovered from tissues obtained at the 96-hour sacrifice of the rats. These results indicate that at least 85% of the administered dose was absorbed and excreted from rats through urine or feces.

Less quantitative estimates of oral absorption have been provided in analytical studies of biphenyl and metabolites in urine and feces from rabbits (Meyer, 1977), guinea pigs (Meyer, 1977), and pigs (Meyer et al., 1976a) following oral administration of single 100-mg/kg doses of unlabeled biphenyl.

Male White Land rabbits and Sff:PIR guinea pigs were given biphenyl (100 mg/kg) by gavage in soy oil, and urine and feces were collected at 24-hour intervals, up to 96 hours after administration (Meyer, 1977). The phenolic metabolites of biphenyl were analyzed as trimethylsilyl (TMS) ethers by combined gas chromatography (GC)/mass spectrometry (MS) (guinea pigs) or GC (rabbits). The biphenyl was hydroxylated to monohydroxylated biphenyls and minor amounts of dihydroxylated derivatives, with the main route of excretion being through the urine in both species and the major metabolite being 4-hydroxybiphenyl. In guinea pigs (n = 3), the mass of identified metabolites in urine collected at 24 or 96 hours post-exposure accounted for 29.5 or 32.9% of the administered dose, respectively. In the first 24 hours, biphenyl and biphenyl metabolites in feces accounted for 20.3% of the dose; most of this (14.3%) was biphenyl, presumably unabsorbed. Bile was collected for 24 hours from another group of two bile-cannulated guinea pigs dosed with 100 mg/kg biphenyl. No unchanged biphenyl was detected in the collected bile, but conjugated mono- and dihydroxy metabolites accounted for about 3% of the administered dose. The results with guinea pigs indicate that at

least 33% of the administered dose was absorbed. In rabbits, urinary metabolites accounted for 49.1% of the dose, with most of this (25.4% on the first day and 15.9% on the second day) eliminated as conjugates. In the first 24 hours, biphenyl and metabolites in feces accounted for 1.6% of the dose with 1.4% being biphenyl. These results indicate that at least 49% of the administered dose was absorbed in rabbits.

Absorption of single oral 100 mg/kg doses of biphenyl (in soy oil or propylene glycol) has also been demonstrated in male and female Danish Landrace pigs weighing 31–35 kg (Meyer et al., 1976b). Metabolites identified in urine collected at four 24-hour intervals after dose administration included mono-, di-, and trihydroxybiphenyls, detected as TMS ethers by GC/MS after enzyme hydrolysis of the samples by β-glucuronidase and sulphatase. Metabolites identified and quantified in 24-hour urine samples accounted for averages of 17.5 and 26.5% of the dose administered in soy oil to two female pigs and in propylene glycol to two male pigs, respectively. Unchanged biphenyl was not detected in the urine samples. Metabolites in urine collected for 96 hours accounted for averages of 27.6 and 44.8% of the doses administered to female and male pigs, respectively. No phenolic metabolites of biphenyl were detected in feces collected for 96 hours. Unchanged biphenyl was not detected in the feces collected from male pigs, but the amount of unchanged biphenyl in feces from the two female pigs accounted for 18.4 and 5% of the administered dose. These results indicate that at least about 28 and 45% of oral 100 mg/kg doses of biphenyl were absorbed in female and male pigs, respectively. It is uncertain if the gender difference was due to vehicle differences or actual gender differences in absorption efficiency.

No animal studies were located examining quantitative aspects of absorption of biphenyl by the respiratory tract or skin.

3.2. DISTRIBUTION

No information was located regarding distribution of absorbed biphenyl in humans and limited animal data are available. Meyer et al. (1976a) orally administered 100 mg/kg [^{14}C]-biphenyl to male albino rats and measured radioactivity in the lung, heart, kidney, brain, spleen, liver, skeletal muscles, peritoneal fat, genital tract, and gastrointestinal tract at 96 hours after dosing. Most of the radioactivity was excreted in urine (84.8%) and feces (7.3%) over the 96-hour period, and only 0.6% of the administered radioactivity remained in the animals at 96 hours: 0.1% was found in peritoneal fat, 0.3% in the gastrointestinal tract (including its contents), 0.1% in skeletal muscles, and 0.1% in the genital tract. Levels of radioactivity in other examined tissues were very low. The results indicate that absorbed biphenyl is not preferentially stored in tissues and is rapidly excreted, principally through the urine.

3.3. METABOLISM

3.3.1. Identification of Metabolites

3.3.1.1. *Results from In Vivo Animal Studies*

No human studies on the in vivo metabolism of biphenyl have been identified. However, the in vivo metabolism of biphenyl has been studied extensively in laboratory animals. These studies have determined that in rats, rabbits, pigs, dogs, mice, and guinea pigs, biphenyl is converted into a range of hydroxylated metabolites (Halpaap-Wood et al., 1981b; Meyer, 1977; Meyer and Scheline, 1976; Meyer et al., 1976a; Meyer et al., 1976b). These metabolites have been detected in urine as both nonconjugated compounds and acidic conjugates.

The derivation of urinary metabolites and their subsequent analysis with GC has resulted in the identification of >10 mono-, di-, and trihydroxybiphenyl metabolites from the urine of rats, pigs, guinea pigs, and rabbits (Meyer, 1977; Meyer and Scheline, 1976; Meyer et al., 1976a; Meyer et al., 1976b). These metabolites have been found as mercapturic acid conjugates and glucuronide conjugates (Millburn et al., 1967). Comparable metabolites have been identified among mammalian species tested, although quantitative differences in metabolite formation are evident among species. A major metabolite in the rat, mouse, guinea pig, rabbit, and pig was reportedly 4-hydroxybiphenyl (Halpaap-Wood et al., 1981b; Meyer, 1977; Meyer and Scheline, 1976). 4,4'-Dihydroxybiphenyl was identified as a major metabolite in the pig (Meyer et al., 1976b) and the rat (Halpaap-Wood et al., 1981b; Meyer and Scheline, 1976), while 3,4-dihydroxybiphenyl was a major urinary metabolite in two strains of mice (Halpaap-Wood et al., 1981b). Table 3-1 reviews the metabolites that have been identified in the excreta and bile of male albino rats given single doses of 100 mg biphenyl/kg, as reported by Meyer and Scheline (1976).

Table 3-1. Metabolites of biphenyl identified in urine, feces, and bile of male albino rats

Metabolite[a]	Urine				Feces	Bile
	Day 1	Day 2	Days 3 + 4	Days 1–4	Day 1	Day 1
Biphenyl	0.1	0.1	ND[b]	0.2	ND	ND
2-Hydroxybiphenyl	0.4	0.5	0.1	1.0	0.3	0.1
3-Hydroxybiphenyl	0.9	0.4	0.3	1.6	0.5	0.5
4-Hydroxybiphenyl	6.8	0.7	0.2	7.7	1.0	1.5
3,4-Dihydroxybiphenyl	0.6	0.2	ND	0.8	ND	0.1
3,4'-Dihydroxybiphenyl	1.5	0.3	0.8	2.6	ND	0.3
4,4'-Dihydroxybiphenyl	9.6	1.7	0.1	11.4	1.8	1.9
2,5-Dihydroxybiphenyl	Trace	ND	ND	Trace	ND	ND
Methoxy-hydroxybiphenyls	0.1	ND	ND	0.1	ND	0.1
Methoxy-dihydroxybiphenyls	0.5	0.3	0.1	0.9	ND	ND
3,4,4'-Trihydroxybiphenyl	1.8	0.9	0.5	3.2	1.1	0.7
Total	22.3	5.1	2.1	29.5	4.7	5.2

[a]Values are percent of administered dose.
[b]ND = not detected.

Source: Meyer and Scheline (1976).

The hydroxylation of biphenyl to produce 2-hydroxybiphenyl is a minor pathway in rats and mice, but is more easily detected in mice than rats (Halpaap-Wood et al., 1981a, b). Following intraperitoneal (i.p.) injection of [^{14}C]-labeled biphenyl (30 mg/kg), the pattern of percentages of radioactivity detected in urinary metabolites showed a relatively greater ability to produce 2-hydroxybiphenyl in mice than rats. In Sprague-Dawley rats, metabolites identified in order of abundance were (with percentage of total urinary radioactivity noted in parentheses): 4,4'-dihydroxybiphenyl (44.5%); 4-hydroxybiphenyl (28.5%); 3,4,4'-trihydroxybiphenyl (8.8%); 3,4'-dihydroxybiphenyl (8.5%); 3,4-dihydroxybiphenyl (5.1%); 3-hydroxybiphenyl (1.8%); and 2-hydroxybiphenyl (1.5%). In DBA/2Tex mice, major identified metabolites were: 4-hydroxy-biphenyl (39.5%); 3,4-dihydroxybiphenyl (30.3%); 4,4'-dihydroxybiphenyl (10.2%); 3,4,4'-trihydroxybiphenyl (6.2%); 3-hydroxybiphenyl (4.3%); and 2-hydroxybiphenyl (4.2%). In rats, 2,3-, 2,4-, and 2,5-dihyroxybiphenyl were detected at trace levels (<0.1%), whereas in mice, these metabolites were detected at levels of 0.3, 0.8, and 0.7%, respectively (Halpaap-Wood et al., 1981b).

No in vivo studies have been identified that directly investigate differential metabolism of biphenyl between males and females of any species. However, studies on urinary crystals and calculi formation and composition after chronic exposure to biphenyl in the diet indicate that male F344 rats are more susceptible than females to the formation of urinary bladder calculi (Ohnishi et al., 2001; Ohnishi et al., 2000a; Ohnishi et al., 2000b). Urinary bladder calculi in

males were predominantly composed of the insoluble potassium salt of 4-hydroxybiphenyl-O-sulphate, whereas the less frequently occurring urinary bladder calculi in females were composed mainly of 4-hydroxybiphenyl and potassium sulphate, hydrolysis products of 4-hydroxybiphenyl-O-sulphate (Ohnishi et al., 2001; Ohnishi et al., 2000a; Ohnishi et al., 2000b). These observations are consistent with observations that male rats have relatively higher urinary potassium concentrations and pH values than female rats, and with the hypothesis that gender differences in these urinary conditions (rather than gender differences in metabolism of biphenyl) may be responsible for the gender differences in urinary calculi formation and the subsequent development of non-neoplastic (hyperplasia) and neoplastic (papillomas and carcinomas) lesions in male, but not female, F344 rats (Umeda et al., 2002; Ohnishi et al., 2001; Ohnishi et al., 2000a; Ohnishi et al., 2000b).

3.3.1.2. *Results from In Vitro Studies with Animal and Human Cells or Tissues*

The metabolism of biphenyl in vitro has been investigated using tissues of human origin, resulting in evidence that the human metabolism of biphenyl is qualitatively similar to, but may be quantitatively different from, rat metabolism. Benford et al. (1981) measured 2-, 3-, and 4-hydroxylation of biphenyl in microsomes prepared from the livers of five rats (sex not identified) and four humans (sex not identified). The reaction products, after solvent extraction and high-performance liquid chromatography (HPLC) quantitation, revealed that 2-hydroxylase in the rat was 35 times higher than in humans, while 3- and 4-hydroxylases in humans were 1.5 and 1.2 times higher than in rats.

The evidence from studies of human tissue samples exposed to biphenyl metabolites in vitro suggests differential Phase II metabolism contingent upon tissue origin. Powis et al. (1988) have shown that *p*-hydroxybiphenyl is conjugated with glucuronic acid and sulphate in human liver and kidney tissue slices. In the liver, glucuronidation was the favored conjugation pathway, while sulphation was favored in the kidney. Powis et al. (1989) also compared Phase I biphenyl metabolism in human (from surgery), dog (mongrel), and rat (male F344) liver slices and primary hepatocytes. It was found that liver slices from all three species had a similar capacity to metabolize biphenyl, ~3.5 nmol biphenyl/minute per g tissue, while hepatocyte preparations from rats had about 4 times the metabolic capacity of dog hepatocytes and about 20 times that of human hepatocytes. Powis et al. (1989) speculated that hepatocytes from dog and human liver slices may have experienced more damage during isolation than rat hepatocytes.

A study of the sulphation of biphenyl metabolites in human surgical tissue samples was conducted by Pacifici et al. (1991). Tissue samples of various types (liver, intestinal mucosa, lung, kidney, bladder, and brain) were obtained from surgeries of patients of both sexes between the ages of 49 and 76 years of age (each patient contributed only one tissue type, so that within-patient organ comparisons were not made). The tissues were homogenized, filtered, and centrifuged at 12,000 and 105,000 g to obtain supernatants to study sulphation of biphenyl

metabolites, specifically 2-, 3-, and 4-hydroxybiphenyl. Sulphotransferase activity for each of these substrates was detected in all tissues studied, although marked tissue dependence was observed, with the highest activity found in the liver and the lowest in the brain. The Michaelis constant (K_m) of sulphotransferase was dependent on the substrate, but not on tissue type, with K_m varying over a 500-fold range. The highest values of K_m were found with 4-hydroxybiphenyl and the lowest were found with 3-hydroxybiphenyl.

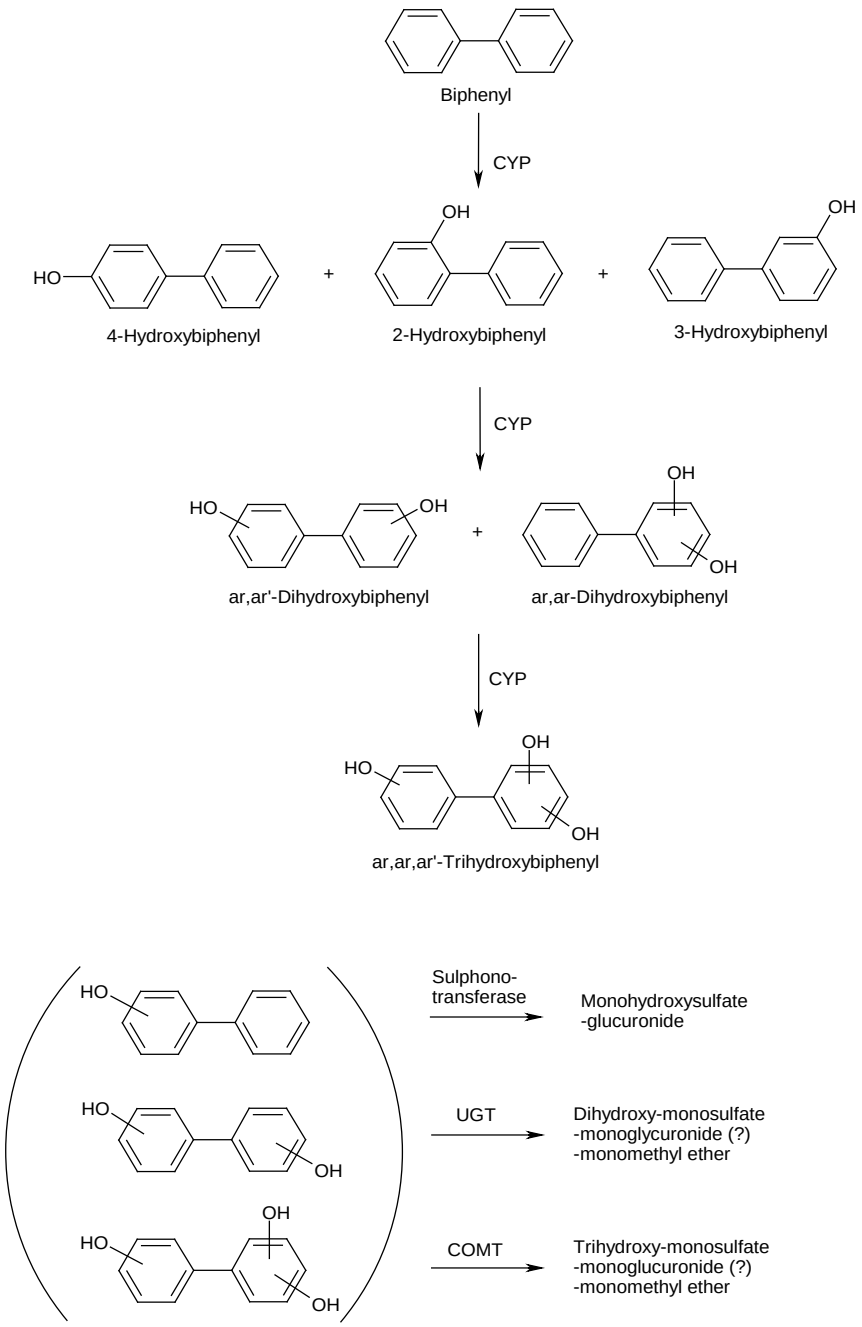
Several studies of biphenyl metabolism with in vitro animal systems support the findings from the in vivo urinary metabolite investigations that: (1) a range of hydroxylated biphenyl metabolites are formed, (2) 4-hydroxybiphenyl is a major metabolite, and (3) hydroxylated biphenyl metabolites are conjugated to glucuronic acid or sulphate. Wiebkin et al. (1984; 1976) reported that isolated rat and hamster hepatocytes metabolized biphenyl primarily to 4-hydroxybiphenyl and also to 4,4'-hydroxybiphenyl, both of which were then conjugated. A small amount of 2-hydroxybiphenyl was produced. When 4-hydroxybiphenyl was incubated with the hepatocytes, it was hydroxylated to 4,4'-dihydroxybiphenyl. Pretreatment of the animals with either 5,6-benzoflavone or phenobarbital had little effect on the conjugate formation rate in the in vitro experiment. Bianco et al. (1979) reported that rat hepatic microsomes metabolize biphenyl to 4-, 2-, and 3-hydroxybiphenyl, which are conjugated to form glucuronides and sulphates. The 4-hydroxybiphenyl isomer was the major metabolite. The formation of 4-hydroxybiphenyl as a major metabolite in the hamster, mouse, and rabbit was confirmed by Billings and McMahon (1978) 2-Hydroxybiphenyl and 3-hydroxybiphenyl were detected in a lower amount in a ratio of 2:1 by hamster and rabbit microsomes, and in a 1:1 ratio by mouse microsomes. In contrast, almost all hydroxylation of biphenyl in rat microsomes gave rise to 4-hydroxybiphenyl.

3.3.2. Metabolic Pathways

3.3.2.1. *Description of Metabolic Scheme and Enzymes Involved*

Burke and Bridges (1975) suggested that biphenyl metabolism is mediated by cytochrome P-450 (CYP) monooxygenases. Evidence of an arene oxide intermediate, which may participate in binding to cellular macromolecules, was reported by Billings and McMahon (1978). Support for CYP metabolism of biphenyl was provided by Halpaap-Wood et al. (1981a, b), who reported that greater amounts of hydroxybiphenyls were obtained in in vitro assays using liver homogenates when rats were treated first with β-naphthoflavone, 3-methylcholanthrene, or Aroclor 1254, which are known CYP inducers. In C57BL/6Tex mice, CYP induction with β-naphthoflavone led to relatively greater amounts of urinary excretion of 2-hydroxybiphenyl, compared with uninduced mice, whereas pretreatment with β-naphthoflavone led to increases in urinary excretion of 2-, 3-, and 4-hydroxybiphenyl in Sprague-Dawley rats and was without influence on the pattern of hydroxybiphenyl metabolites in DBA/2Tex mice (Halpaap-Wood et al., 1981b).

Figure 3-1 details combined evidence from the Halpaap-Wood et al. (1981a, b) and Meyer and Scheline (1976b) studies on the metabolic pathways of biphenyl. While sulphates and glucuronides are formed on all three metabolic levels illustrated, only monosulphates and monoglucuronides are identified. Monomethyl ethers are formed from dihydroxy and trihydroxy metabolites alone. Glucuronides at the dihydroxy and trihydroxy levels are additionally labeled with a question mark to suggest that, while these metabolites are likely, they have not been identified.



ar = aryl group; COMT = catechol-O-methyltransferase; UGT = uridine diphosphate glucuronosyl transferase; question marks denote tentative metabolites (see text).

Sources: Halpaap-Wood et al. (1981a, b); Meyer and Scheline (1976b).

Figure 3-1. Schematic presentation of the metabolic pathways of biphenyl.

The metabolic scheme in Figure 3-1 does not include the possible redox cycling of 2,5-dihydroxybiphenyl (also known as phenylhydroquinone), which involves CYP-mediated cycling between phenylhydroquinone and phenylbenzoquinone leading to the generation of reactive oxygen species (ROS) (Balakrishnan et al., 2002; Kwok et al., 1999). This pathway is thought to play a role in the carcinogenic effect of 2-hydroxybiphenyl (also known as *ortho*-phenylphenol), a broad spectrum fungicide that, like biphenyl, induces urinary bladder tumors in chronically exposed male rats (Kwok et al., 1999). Free 2,5-dihydroxybiphenyl and its glucuronide or sulphate conjugates are readily detected in the urine of rats exposed to 2-hydroxybiphenyl, and the formation of 2,5-dihydoxybiphenyl and phenylbenzoquinone is the principal metabolic pathway for 2-hydroxybiphenyl in the rat, especially at high exposure levels associated with urinary bladder tumor formation (Kwok et al., 1999; Morimoto et al., 1989; Nakao et al., 1983; Reitz et al., 1983; Meyer and Scheline, 1976). In contrast, the formation of 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl is the principal metabolic pathway for biphenyl in rats and mice, and 2,5-dihydroxybiphenyl was not detected, or only detected at trace levels, in the urine of rats exposed to 100 mg biphenyl/kg (Meyer et al., 1976b) (see Table 3-1). In mice exposed to i.p. doses of [^{14}C]-biphenyl (30 mg/kg), radioactivity in 2-hydroxybiphenyl and 2,5-dihydroxybiphenyl in the urine accounted for only about 5% of the total radioactivity detected in urinary metabolites (Halpaap-Wood et al., 1981b).

3.3.3. Regulation of Metabolism, Sites of Metabolism, and Relationships to Toxic Effects

3.3.3.1. *Evidence for Induction of Phase I and II Enzymes*

No studies of Phase I or II enzyme induction using liver microsomes of human origin were identified. However, a number of studies have been conducted in rodents to investigate the induction of Phase I enzymes that catalyze biphenyl hydroxylation. For example, Creaven and Parke (1966) reported that pretreatment of weanling Wistar rats or ICI mice with phenobarbital [an inducer of CYP3A4, 2B6, and 2C8 as reported by Parkinson and Ogilvie (2008)] or 3-methylcholanthrene [an inducer of CYP1A2 as reported by Parkinson and Ogilvie (2008)] increased NADPH-dependent activities of liver microsomes to produce 2-hydroxybiphenyl and 4-hydroxybiphenyl from biphenyl to varying degrees depending on the inducer. Haugen (1981) reported that pretreatment of male CD rats with phenobarbital or 3-methylcholanthrene increased NADPH-dependent activities of liver microsomes to produce 2-, 3-, and 4-hydroxybiphenyl from biphenyl, again to varying degrees depending on the inducer. Stuehmeier et al. (1982) reported that phenobarbital pretreatment of male C57BL/6JHan mice induced liver microsomal activities to produce 4-hydroxybiphenyl, but not 2-hydroxybiphenyl, from biphenyl, whereas 3-methylcholanthrene induced activities for both 4- and 2-hydroxylation of biphenyl. Halpaap-Wood et al. (1981b) reported that pretreatment of male Sprague-Dawley rats with β-naphthoflavone [an inducer of CYP1A2 as reported by Parkinson and Ogilvie (2008); also known as 5,6-benzoflavone] enhanced the urinary excretion of 2-, 3-, and 4-hydroxybiphenyl,

3,4-dihydroxybiphenyl, and 3,4,4'-trihydroxybiphenyl following i.p. administration of 30 mg biphenyl/kg body weight. In contrast, pretreatment of male C57BL/6Tex mice with β-naphthoflavone did not increase the overall urinary excretion of biphenyl metabolites following i.p. administration of 60 mg biphenyl/kg, but shifted the principal metabolite from 4-hydroxybiphenyl to 2-hydroxybiphenyl and 2,5-dihydroxybiphenyl (Halpaap-Wood et al., 1981b). Wiebkin et al. (1984) reported that β-naphthoflavone pretreatment of male Lewis rats or male Syrian golden hamsters induced biphenyl hydroxylation activities in freshly isolated pancreatic acinar cells or hepatocytes. From these observations and examination of patterns of inhibition of biphenyl hydroxylation activities by CYP inhibitors (e.g., α-naphthoflavone and 1-benzyl-imidazole) under non-induced and induced conditions (Haugen, 1981), it is apparent that multiple CYP enzymes (e.g., CYP1A2 and CYP3A4) are likely involved in biphenyl hydroxylation. However, no studies were located that used more modern techniques (such as CYP knockout mice) to identify the principal CYP enzymes involved in the initial hydroxylation of biphenyl or the formation of the dihydroxy- or trihydroxybiphenyl metabolites.

Several animal studies were located examining the possible coordinated induction of Phase I enzymes with Phase II enzymes catalyzing the conjugation of hydroxylated biphenyl metabolites to sulphate or glucuronic acid. Hepatocytes from rats (strain and sex were not noted) pretreated with the CYP inducers, phenobarbital or 3-methylcholanthrene, produced glucuronide and sulphate conjugates of 4-hydroxybiphenyl when incubated with biphenyl (Wiebkin et al., 1978). Glucuronide conjugates were predominant under these "CYP-induced" conditions, whereas hepatocytes from non-induced control rats produced predominant sulphate conjugates of 4-hydroxybiphenyl. These results suggest that induction (or possibly activation) of glucuronidation enzymes may be coordinated with the induction of CYP enzymes. In contrast, pretreatment of male Lewis rats with β-naphthoflavone (an inducer of CYP1A2) did not enhance activities of freshly isolated pancreatic acinar cells to conjugate 4-hydroxybiphenyl with sulphate or glucuronic acid, but the influence of this pretreatment on the conjugation capacity of hepatocytes was not examined in this study (Wiebkin et al., 1984). In another study, uridine diphosphate glucuronosyl transferase (UGT) activities with 1-naphthol or 3-hydroxy-benzo[a]pyrene as substrates were higher in liver microsomes from male Wistar rats pretreated with Aroclor 1254 (an inducer of several CYP enzymes) or phenobarbital, respectively, compared with microsomes from control rats without pretreatment with CYP inducers (Bock et al., 1980). Although Bock et al. (1980) measured UGT activities in microsomes from several tissues from non-induced rats with 4-hydroxybiphenyl as a substrate, no comparisons between induced and non-induced conditions were made using 4-hydroxybiphenyl as substrate. Paterson and Fry (1985) reported that hepatocytes or liver slices from male Wistar rats pretreated with β-naphthoflavone showed decreased rates of glucuronidation of 4-hydroxybiphenyl, compared with hepatocytes or liver slices from rats without β-naphthoflavone pretreatment. Results from this database provide equivocal evidence that the induction of Phase I enzymes catalyzing the

hydroxylation of biphenyl may be coordinated with induction of Phase II enzymes catalyzing glucuronidation of hydroxylated biphenyl metabolites.

3.3.3.2. *Demonstrated Tissue Sites of Metabolism*

CYP enzymes catalyzing hydroxylation of biphenyl and other substrates are present in most, if not all, mammalian tissues, but the highest levels of activities are normally found in liver (Parkinson and Ogilvie, 2008). In a study of male Sprague-Dawley rats, CYP content was 20–40-fold higher in the microsomes from liver than from lung, although biphenyl-4-hydrolase activity was only 1.7-fold higher in the microsomes from liver than from lung (Matsubara et al., 1974). Wiebkin et al. (1984) observed 200- and 1,000-fold higher rates of biphenyl metabolism in 5,6-benzoflavone-pretreated hepatocytes compared to similarly treated pancreatic acinar cells from male Lewis rats and Syrian golden hamsters, respectively.

Activities for enzymes catalyzing the conjugation of hydroxybiphenyls and other hydroxylated aromatic compounds with glucuronic acid or sulphate have been detected in a number of mammalian tissues, and, similar to CYP, the highest levels are found in the liver (Parkinson and Ogilvie, 2008). Available data for conjugation activities with hydroxybiphenyls in various mammalian tissues are consistent with this concept. Sulphotransferase activities with 2-, 3-, or 4-hydroxybiphenyl as substrates in microsomes from several human tissues showed an approximate 100- to 500-fold range with the following order: liver > ileum > lung > colon > kidney > bladder > brain (Pacifici et al., 1991). UGT activities with 4-hydroxybiphenyl as substrate in microsomes from several male Wistar rat tissues showed the following order: liver > intestine > kidney > testes ≈ lung; activities were below the limit of detection in microsomes from skin and spleen (Bock et al., 1980).

3.4. ELIMINATION

No studies were located on the route or rate of elimination of biphenyl in humans, but results from studies of orally exposed animals indicate that absorbed biphenyl is rapidly eliminated from the body, principally as conjugated hydroxylated metabolites in the urine.

The most quantitative data on the routes and rates of elimination come from a study of rats following administration of radiolabeled biphenyl (Meyer et al., 1976b). Urine collected for 24 hours after the oral administration of 100 mg/kg [^{14}C]-labeled biphenyl in soy oil to male albino rats contained 75.8% of the administered radioactivity, compared with 5.8% detected in feces collected in the same period. Ninety-six hours after dose administration, <1% of the administered radioactivity remained in tissues, 84.8% was in collected urine, 7.3% was in feces, and 0.1% was in collected expired air (Meyer et al., 1976a). Although chemical identity analysis of fecal radioactivity was not conducted by Meyer et al. (1976a) (results from GC/MS analyses of bile collected from bile-cannulated rats given single 100 mg/kg doses of unlabeled biphenyl indicate that biliary excretion of metabolites represents a minor pathway of elimination (Meyer

and Scheline, 1976). In bile collected for 24 hours, unchanged biphenyl was not detected and conjugated metabolites accounted for 5.2% of the administered dose; in contrast, conjugated metabolites of biphenyl in 24-hour urine accounted for 22.3% of the dose (Meyer and Scheline, 1976).

Supporting evidence for the importance of urinary elimination of conjugated metabolites is provided by the results of other studies, which analyzed biphenyl and biphenyl metabolites by GC/MS or GC in urine and feces collected from rabbits (Meyer, 1977), guinea pigs (Meyer, 1977), and pigs (Meyer et al., 1976b) following oral administration of 100 mg/kg doses of unlabeled biphenyl. In 24-hour urine samples, unchanged biphenyl was not detected, and total metabolites accounted for averages of 25.4% of the administered dose in rabbits, 31.3% in guinea pigs, 17.5% in female pigs, and 26.4% in male pigs. As in rats, biliary excretion represents a minor elimination pathway in guinea pigs and rabbits; metabolites detected in bile collected for 24 hours from bile-cannulated guinea pigs accounted for 3.3% of the administered dose, but for only 0.3% of the dose in bile collected for 7 hours from a rabbit given 100 mg/kg biphenyl (Meyer, 1977). Neither unchanged biphenyl nor hydroxylated biphenyl metabolites were detected in bile collected from a bile-cannulated pig for 24 hours after administration of 100 mg/kg biphenyl (Meyer et al., 1976b).

No studies were located examining quantitative aspects of elimination in animals following inhalation or dermal exposure to biphenyl.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS

No studies were located on the development of PBPK models for biphenyl in animals or humans.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS

Limited human data include assessments of workers exposed to biphenyl during production of biphenyl-impregnated fruit wrapping paper at one mill in Finland (Seppalainen and Hakkinen, 1975; Häkkinen et al., 1973; Häkkinen et al., 1971) and another mill in Sweden (Wastensson et al., 2006).

A case report of a 46-year-old female who worked at a fruit-packing facility over a 25-year period where biphenyl-impregnated paper was used presented with hepatomegaly, neutrophilic leukocytosis, clinical chemistry findings indicative of hepatic perturbation, and liver biopsy indicative of chronic hepatitis (Carella and Bettolo, 1994). Following cessation of work in citrus packing, serum enzymes returned to normal, suggesting that occupational exposure to biphenyl may have been the principal etiological factor.

Häkkinen and colleagues assessed the health of paper mill workers exposed to biphenyl during the production of biphenyl-impregnated paper used to wrap citrus fruits. In 1959, workers complained about a strong odor and irritation to the throat and eyes. Air measurements made at various locations within the facility in June of 1959 resulted in estimated average biphenyl concentrations of 4.4–128 mg/m^3 (Table 4-1). In 1969, a 32-year-old worker at the facility, who had worked for 11 years in the oil room where biphenyl levels were particularly high, became ill. Despite aggressive medical intervention, the patient grew worse and died. Key features at autopsy included necrosis of most liver cells, severe, but unspecified changes in the kidneys, degeneration of the heart muscles, hyperactive bone marrow, and edematous changes in the brain (Häkkinen et al., 1973; 1971). Subsequent measurements of biphenyl in the workplace air (January 1970) resulted in estimated average concentrations ranging from 0.6 to 123 mg/m^3 (Table 4-1). Measurements taken in both 1959 and 1971 indicated that biphenyl air concentrations at multiple work areas greatly exceeded the current American Conference of Governmental Industrial Hygienists (ACGIH, 2001) threshold limit value (TLV) of 0.2 ppm (1.3 mg/m^3). In the location where biphenyl was mixed with paraffin oil (the oil room), biphenyl occurred both as a vapor and as a dust, suggesting the possibility of both dermal and inhalation exposures.

Table 4-1. Biphenyl concentrations in the air of a Finnish paper mill producing biphenyl-impregnated fruit wrapping paper

Sampling center locations	Average concentrations (mg/m^3)	
	June 1959	January 1970
Paper mill hall		
In front of paper reel	17.9	7.2
Behind impregnating roller	128.0	64.0
Near paper machine	7.2	1.5
Near rolling machine	4.4	0.6
Oil-room		
Near measuring container	19.5	3.5
Above measuring container (lid open)	No data	123.0
Near mixing container	No data	15.5
During addition of biphenyl to mixing container	No data	74.5

Source: Häkkinen et al. (1973).

Thirty-one male workers at the Finnish facility engaged in the biphenyl-impregnation process and two other workers exposed to biphenyl were included in the study. Common complaints among these workers included fatigue, headache, gastrointestinal discomfort, numbness and aching of the limbs, and general fatigue; laboratory tests revealed elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (which can indicate inflammation or damage to liver cells) in 10 of the 33 workers (Häkkinen et al., 1973). Eight of the 33 workers were admitted to the hospital for further examination, including liver biopsy. The majority of the 33 workers were subjected to neurophysiological examinations, including electroencephalography (EEG) and electroneuromyography (ENMG, consisting of nerve conduction velocity and electromyographic [EMG] tests). Seppäläinen and Häkkinen (1975) published the most comprehensive results of the neurophysiological examinations. In all, 24 subjects (including the 8 hospitalized workers) underwent neurophysiological examinations. Exposure to biphenyl was terminated immediately following the initial neurophysiological examinations, and 11 and 7 of these subjects were retested 1 and 2 years later, respectively.

EEG results. At initial examination, 10 of the 24 workers had abnormal EEGs, which included diffuse slow wave abnormalities (6 cases), lateral spike and slow wave discharges (2 cases), posterior slowing only (1 case), and mild slow wave abnormality in the right temporal area (1 case). Six subjects exhibited unusual distribution of alpha rhythm with alpha activity also prominent in the frontal areas. Four of the subjects exhibited no EEG abnormalities. In general, the EEG results observed at initial examination were qualitatively similar in the 11 subjects reexamined 1 year later. Exceptions included additional diffuse slow wave abnormalities in the two subjects initially exhibiting only spike and wave discharges and the disappearance of the one

case of mild temporal local abnormality. There was no discernable improvement in the EEGs of the seven subjects reexamined after 2 years.

ENMG results. As shown in Table 4-2, the 24 biphenyl-exposed workers exhibited no significant differences in mean maximal motor conduction velocity (MCV) relative to those of a control group consisting of 60 healthy Finnish males, but significantly ($p < 0.001$) slower mean conduction velocity of the slowest motor fibers (CVSF) of the ulnar nerves. Results at the 1-year follow up of 11 of the biphenyl-exposed workers revealed no significant changes in initial conduction velocity measures, but at the 2-year reexamination of 7 of the 11 subjects, the MCVs of the median and deep peroneal nerves were significantly slower ($p < 0.02$ and $p < 0.01$, respectively) compared to the initial measurements. Abnormal EMGs among the biphenyl-exposed workers included diminished numbers of motor units on maximal muscle contraction (10 subjects) and fibrillations in some muscles (7 subjects). Workers exhibiting abnormal EMGs typically displayed slowing of some nerve conduction velocities as well. Of those 11 subjects undergoing repeat ENMG examination after 1 year, 5 subjects showed an increased level of ENMG abnormality, while 4 remained unchanged and 2 had diminished abnormalities. At the end of 2 years, three of seven subjects displayed diminished ENMG abnormalities, three of seven were unchanged, and one of seven had the abnormality increased.

Table 4-2. Nerve conduction velocities of 24 persons exposed to biphenyl: comparison with 60 unexposed males

Nerve	Biphenyl group (mean ± SD)	Control group (mean ± SD)	*t*-test
Median			
MCV	57.7 ± 6.3	58.0 ± 3.8	Not significant
Ulnar			
MCV	56.3 ± 4.6	56.6 ± 4.0	Not significant
CVSF	41.4 ± 5.2	45.5 ± 3.2	$p < 0.001$
Deep peroneal			
MCV	50.2 ± 5.4	50.3 ± 3.5	Not significant
CVSF	37.7 ± 3.9	38.2 ± 5.6	Not significant
Posterior tibial			
MCV	43.4 ± 3.9	42.4 ± 4.7	Not significant

SD = standard deviation

Source: Seppäläinen and Häkkinen (1975).

Seppäläinen and Häkkinen (1975) noted that subjects often exhibited signs of dysfunction in both the peripheral nervous system, as evidenced by abnormal ENMGs, and the central nervous system, as evidenced by abnormal EEGs and abnormal distribution of alpha activity. Only five subjects (four men and the only woman in the biphenyl-exposed group) were found to

have completely normal neurophysiological records. The authors interpreted their data to indicate that biphenyl can attack the nervous system at different levels, the sites of greatest vulnerability being the brain and peripheral nerves. Compound-related anomalies in nerve conduction, EEG, and ENMG signals, while small, were consistent with the persistence of incapacity and the incidence of subjective symptoms. At a facility manufacturing biphenyl-impregnated paper in Sweden, a cluster of five cases of Parkinson's disease (PD) among the employees was investigated (Wastensson et al. 2006). Since, according to the report, the expected prevalence of PD would be less than 0.9 cases from the 255 employees at the facility (relative risk [RR] 5.6 [95% confidence interval 1.9–13]), it was suspected that the elevated PD at the facility may have been related to biphenyl exposure. Four of the subjects worked in the vicinity of a rewinder/dryer, while the fifth attended to another rewinder. Although no ambient biphenyl levels were available for the subjects' work space, it was thought likely that the level of biphenyl in air would be greater than the existing TLV of 1.3 mg/m^3 (0.2 ppm) based on measurements at a Finnish paper mill with similar production practices (Häkkinen et al., 1973). Two subjects may have been exposed to higher levels of biphenyl than the others when they created the paraffin oil/biphenyl mixture.

In addition to comparing existing PD cases to national trends, Wastensson et al. (2006) examined the medical records of 222 former employees who had died. Nine cases of PD were found among the decedents, compared with 4.3 cases of PD expected from data on the lifetime risk of developing PD in the general population. This comparison yielded an RR of 2.1, with a 95% confidence interval of 0.96–4.0.

Clinical features and exposure data for the five living subjects, all of whom were diagnosed with PD by a neurologist at a local hospital, are summarized in Table 4-3. With one exception, the patients were in comparatively good health on initial diagnosis. The exception was a 53-year-old male who had diabetes mellitus and withdrew from the study before his neurological condition could be confirmed. Assuming that the diagnoses of PD were valid, the central issue is whether these data indicate a causal relationship between PD and exposure to biphenyl. Wastensson et al. (2006) discussed this issue in the context of other studies that have pointed to a possible cause-and-effect relationship between pesticide exposure and PD, but were unable to draw any firm conclusions from their limited data.

Table 4-3. Exposure data and clinical features for five PD patients with occupational exposure to biphenyl

	Case				
	1	**2**	**3**	**4**	**5**
Exposure data					
Age	63	63	58	54	63
Workplace	PM3	PM3	PM4	PM3	PM3
Years of exposure[a]	12	4	9	4	2
Age at onset of exposure	19	26	17	18	21
Age at onset of symptoms	52	55	44	51	55
Clinical features					
Resting tremor	+	+	+	+	+
Cogwheel rigidity	+	+	+	–	+
Bradykinesia	+	+	+	+	–
Positive response to levodopa[b]	+	+	+	+	+

[a]Exposure to biphenyl about one-third of each year.
[b]All five patients improved with levodopa, which is a medication for PD.

PM = paper mill

Source: Wastensson et al. (2006)

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Available oral data for biphenyl include two well-designed two-year chronic toxicity and carcinogenicity studies, one in F344 rats (Umeda et al., 2002) and one in BDF_1 mice (Umeda et al., 2005). Increased incidence of urinary bladder transitional cell papillomas and carcinomas, associated with the formation of urinary bladder calculi, occurred in male, but not female, F344 rats at the highest tested dietary concentration, 4,500 ppm, but were not found at lower exposure levels of 1,500 or 500 ppm. Non-neoplastic kidney lesions (simple transitional cell hyperplasia in the renal pelvis and hemosiderin deposits) were found in female F344 rats at biphenyl dietary concentrations ≥1,500 ppm (Umeda et al., 2002). Several other rat studies provide supporting evidence that the kidney and other urinary tract regions are critical targets for biphenyl in rats (Shiraiwa et al., 1989; Ambrose et al., 1960; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953). In BDF_1 mice, increased incidence of liver tumors (hepatocellular adenomas and carcinomas) and non-neoplastic effects on the kidney (mineralization) and liver (increased activities of plasma ALT and AST) were found in females exposed to biphenyl dietary concentrations of 2,000 or 6,000 ppm (Umeda et al., 2005). In contrast, no carcinogenic responses or noncancer adverse effects were found in female ddY mice exposed to 5,000 ppm biphenyl in the diet for 2 years (Imai et al., 1983) or in $B6C3F_1$ and $B6AKF_1$ mice exposed to 517 ppm biphenyl in the diet for 18 months (Innes et al., 1969; NCI, 1968).

No chronic inhalation toxicity studies in animals are available. In subchronic inhalation toxicity studies, respiratory tract irritation and increased mortality following exposure to dusts of biphenyl (7 hours/day, 5 days/week for up to about 90 days) were reported in mice exposed to 5 mg/m^3 and in rats exposed to 300 mg/m^3, but not in rabbits exposed to 300 mg/m^3 (Deichmann et al., 1947; Monsanto, 1946). Congestion or edema of the lung, kidney, and liver, accompanied by hyperplasia with inflammation of the trachea, was found in CD-1 mice exposed to biphenyl vapors at 25 or 50 ppm (158 or 315 mg/m^3) for 13 weeks (Sun, 1977a).

Study descriptions for all available subchronic and chronic toxicity and carcinogenicity studies follow.

4.2.1. Oral Exposure

4.2.1.1. *Subchronic Toxicity*

Twenty-one-day-old female Long-Evans rats (8/group) were exposed to 0, 0.01, 0.03, or 0.1% biphenyl in the diet for 90 days (Dow Chemical Co.). Body weights were monitored 3 times/week, and the weights of the liver, kidneys, adrenals, and spleen were recorded at necropsy. Heart, liver, kidney, spleen, adrenals, pancreas, ovary, uterus, stomach, small and large intestine, voluntary muscle, lung, thyroid, and pituitary from each rat were examined histopathologically (2 rats/group).

Based on U.S. EPA (1988) subchronic reference values for body weight and food consumption in female Long-Evans rats, doses of biphenyl resulting from the dietary levels of 0.01, 0.03, and 0.1% are estimated to have been 10, 30, and 100 mg/kg-day, respectively. There were no significant treatment-related effects on body weight, food consumption, or organ weights. Results of histopathologic examinations were unremarkable. Biphenyl-exposed groups exhibited lower average plasma blood urea nitrogen (BUN) levels than controls (28.2, 25.7, and 26.3 mg percent for low-, mid-, and high-dose groups, respectively, compared to 35.3 mg percent for controls), although the statistical significance of these apparent treatment-related differences was not reported and the biological significance is uncertain.

Six-week-old BDF_1 mice (10/sex/group) were exposed to biphenyl at dietary concentrations of 0, 500, 2,000, 4,000, 8,000, 10,000, or 16,000 ppm for 13 weeks (Umeda et al. 2004b). To overcome possible problems with taste aversion, mice assigned to the 8,000 and 10,000 ppm groups were fed 4,000 ppm dietary biphenyl for the first week and 8,000 or 10,000 ppm for the remaining 12 weeks. Mice designated to receive 16,000 ppm were fed 4,000 ppm dietary biphenyl for the first week, 8,000 ppm for the second week, and 16,000 ppm for the remaining 11 weeks. Animals were checked daily for clinical signs; body weight and food consumption were recorded weekly; organ weights were noted at term; and liver sections were processed for light microscopic examination. Electron microscopy was carried out on liver tissue from one control and one 16,000 ppm female.

Based on U.S. EPA (1988) subchronic default reference values for body weight and food consumption (average values for combined sexes), doses of biphenyl for the dietary concentrations of 500, 2,000, 4,000, 8,000, 10,000, and 16,000 ppm are estimated to have been 93, 374, 747, 1,495, 1,868, and 2,989 mg/kg-day, respectively. A single 16,000 ppm female mouse died during the study; all other mice survived until terminal sacrifice. Final body weights of mice of both sexes in the 8,000, 10,000, and 16,000 ppm groups were significantly lower than gender-matched controls (for males: 83.3, 84.9, and 75.1% of controls; for females: 93.7, 91.6, and 85.8% of controls, respectively). Umeda et al. (2004a) noted that absolute liver weights were significantly higher in 8,000 and 16,000 ppm female mice, but did not include the extent of these increases in the study report. Light microscopic examination of liver specimens from all 16,000 ppm female mice revealed enlarged centrilobular hepatocytes, the cytoplasm of which was filled with numerous eosinophilic fine granules. Upon electron microscopic examination, these eosinophilic granules were identified as peroxisomes, indicative of a peroxisome proliferative effect in the liver of the 16,000 ppm female mice. Evidence of histopathologic liver lesions was not found in females of the 8,000 or 10,000 ppm groups. There were no signs of treatment-related increased liver weight or histopathologic evidence of clearly enlarged hepatocytes in any of the biphenyl-treated groups of male mice. Based on the significant decrease in body weight in both genders, EPA identified 1,495 mg/kg-day as the LOAEL and 747 mg/kg-day as the NOAEL.

4.2.1.2. *Chronic Toxicity and Carcinogenicity*

4.2.1.2.1. *Chronic rat studies*

In a chronic toxicity and carcinogenicity study of F344 rats (50/sex/group), biphenyl was administered in the diet for 2 years at concentrations of 0, 500, 1,500, or 4,500 ppm (Umeda et al., 2002). All animals were examined daily for clinical signs; body weights and food intake were determined once a week for the first 14 weeks and every 4 weeks thereafter. Urinalysis was performed on all surviving rats during week 105. Upon necropsy, weights of all major organs were recorded; all major organs and tissues were subjected to histopathologic examination.

The study report included a plot of mean body weights during the 2-year study, but did not include food consumption data. Estimated doses, therefore, were calculated using time-weighted average (TWA) body weights from the graphically-depicted data (Umeda et al., 2002 Figure 1) and U.S. EPA (1988) chronic reference values for food consumption in F344 rats. The resulting estimated doses for the 500, 1,500, and 4,500 ppm exposure groups were 36.4, 110, and 378 mg/kg-day, respectively, for males and 42.7, 128, and 438 mg/kg-day, respectively, for females. The study authors reported significantly lower mean body weights among 4,500 ppm rats of both sexes compared to their respective controls. Mean body weights of 4,500 ppm male and female rats were lower than those of controls throughout most of the study period and were approximately 20% lower than respective controls at terminal sacrifice. There was no significant

effect on mean body weights of 500 or 1,500 ppm males or females. Survival of low- and mid-dose male and female rats was not significantly different from controls. The study authors reported that 3/50 of the 4,500 ppm female rats died after 13–26 weeks of biphenyl exposure and attributed the deaths to marked mineralization of the kidneys and heart. However, they also indicated that survival of this group was not adversely affected thereafter. Significantly decreased survival was noted only for the group of 4,500 ppm male rats, 19/50 of which died prior to terminal sacrifice. The first death occurred around treatment week 36; this rat exhibited urinary bladder calculi. Survival data for the other groups were not provided. Evidence of hematuria was first noted in 4,500 ppm male rats around week 40 and was observed in a total of 32/50 of the 4,500 ppm males during the remainder of the treatment period; 14 of these rats appeared anemic. Hematuria and bladder tumors were primarily considered as causes of death among the 4,500 ppm males (n = 19) that died prior to terminal sacrifice. Urinalysis performed during the final treatment week revealed significantly increased urinary pH in the 31 remaining 4,500 ppm male rats (pH of 7.97 versus 7.66 for controls; $p < 0.05$); occult blood was noted in the urine of 23 of these males. Urine samples in 10/37 surviving 4,500 ppm females tested positive for occult blood. Significant increases in relative kidney weights of 1,500 and 4,500 ppm males and females and absolute kidney weights of 4,500 ppm males were reported, but actual data were not presented.

Gross pathologic examinations at premature death or terminal sacrifice revealed the presence of calculi in the bladder of 43/50 of the 4,500 ppm males and 8/50 of the 4,500 ppm females (Table 4-4); these lesions were not seen in 500 or 1,500 ppm male or female rats. The bladder calculi in the male rats were white, yellow, brown, gray, and black in color, ranged from 0.3 to 1.0 cm in size, and exhibited triangular, pyramidal, cuboidal, and spherical shapes. The bladder calculi in the female rats were white and yellow in color, of uniform spheroidal shape, and similar in size to those of the male rats. Forty-one of the 4,500 ppm male rats exhibited polyp-like or papillary nodules protruding into the lumen from the bladder wall; bladder calculi were noted in 38 of these males. Four of the eight calculi-bearing 4,500 ppm female rats also exhibited thickening of the bladder wall. It was noted that 30/32 of the 4,500 ppm male rats with hematuria also exhibited kidney or urinary bladder calculi.

Table 4-4. Incidences of urinary bladder lesions in male and female F344 rats exposed to biphenyl in the diet for 2 years

	Males (n = 50)				Females (n = 50)			
Dietary concentration (ppm)	**0**	**500**	**1,500**	**4,500**	**0**	**500**	**1,500**	**4,500**
Calculated dose (mg/kg-d)	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Lesion								
Transitional cell								
Simple hyperplasia[a]	0	0	0	12[b]	0	0	1	1
Nodular hyperplasia[a]	0	0	0	40[b]	1	0	0	5[c]
Papillary hyperplasia[a]	0	0	0	17[b]	0	0	0	4
Combined	0	0	0	45	1	0	1	10[b]
Papilloma	0	0	0	10[b]	0	0	0	0
Carcinoma	0	0	0	24[b]	0	0	0	0
Papilloma or carcinoma (combined)	0	0	0	31[b]	0	0	0	0
Squamous cell								
Metaplasia[a]	0	0	0	19[b]	0	0	0	4
Hyperplasia[a]	0	0	0	13[b]	0	0	0	1
Papilloma or carcinoma (combined)	0	0	0	1	0	0	0	0
Inflammatory polyp[a]	0	0	0	10[b]	0	0	0	0
Calculi	0	0	0	43[b]	0	0	0	8[b]

[a]The number is the sum of animals with severity grades of slight, moderate, marked, or severe.
[b]Significantly different from control group ($p < 0.01$) according to Fisher's exact test.
[c]Significantly different from control group ($p < 0.05$) according to Fisher's exact test.

Source: Umeda et al. (2002)

Histopathologic examinations at death or terminal sacrifice revealed no indications of biphenyl-induced tumors or tumor-related lesions in organs or tissues other than those associated with the urinary tract. As shown in Table 4-4, neoplastic and nonneoplastic lesions of the urinary bladder were essentially limited to the 4,500 ppm rats and predominantly the males. Only 4,500 ppm male rats exhibited papilloma (10/50) or carcinoma (24/50) of transitional cell epithelium, three of which exhibited both papilloma and carcinoma. Most of the transitional cell carcinomas (20/24) projected into the lumen, and the tumor cells invaded the entire body wall. Bladder calculi were found in all 24 males with transitional cell carcinoma and 8/10 of the males with transitional cell papilloma. Among noncancerous responses in the bladder, simple, nodular, and papillary hyperplasias were evident in 4,500 ppm animals. These hyperplasias developed in the focal area of the bladder epithelium. Simple hyperplasia occurred less frequently than nodular and papillary hyperplasias; furthermore, simple hyperplasia was almost always accompanied by either nodular or papillary hyperplasia in the 4,500 ppm males. Ten of the 4,500 ppm males had polyps in the bladder epithelium, which were composed of spindle fibers

proliferated around transitional epithelial cells accompanied by inflammatory infiltration of submucosal bladder epithelium. Squamous metaplasia was noted on the surface of the polyps, which were found at different loci than the bladder tumors.

Table 4-5 summarizes the incidences of lesions of the ureter and kidney in the male and female rats. The incidence of simple transitional cell hyperplasia in the ureter was greater in the 4,500 ppm males than the 4,500 ppm females. Other responses, such as mineralization of the corticomedullary junction, were increased over controls to a greater extent in males compared to females. In the renal pelvis, simple and nodular hyperplasia was frequently observed in 4,500 ppm males and 500 and 1,500 ppm females. Responses such as papillary necrosis, infarct, and hemosiderin deposition occurred predominantly in exposed females.

Table 4-5. Incidences of ureter and kidney lesions in male and female F344 rats exposed to biphenyl in the diet for 2 years

	Males (n = 50)				Females (n = 50)			
Dietary concentration (ppm)	**0**	**500**	**1,500**	**4,500**	**0**	**500**	**1,500**	**4,500**
Calculated dose (mg/kg-d)	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Response								
Ureter								
Transitional cell simple hyperplasia	1	0	0	8[a]	0	0	0	2
Transitional cell nodular hyperplasia	0	0	0	1	0	0	0	0
Dilatation	0	0	0	14[a]	0	0	0	6[b]
Kidney								
Renal pelvis								
Transitional cell simple hyperplasia	6	8	5	19[c]	3	5	12[c]	25[a]
Transitional cell nodular hyperplasia	0	1	1	21[a]	0	0	1	12[a]
Squamous metaplasia	0	0	0	2	0	0	0	0
Mineralization	9	6	10	18[b]	12	12	18	27[a]
Desquamation	1	0	0	11[a]	0	0	0	2
Calculi	0	0	0	13[a]	0	0	0	3
Other								
Mineralization of corticomedullary junction	0	0	0	10[a]	21	2	26	18
Mineralization of papilla	9	9	14	23[c]	2	6	3	12[a]
Papillary necrosis	0	0	0	7[d]	0	0	0	23[a]
Infarct	0	0	0	0	1	0	0	8[c]
Hemosiderin deposits	0	0	0	0	4	8	22[a]	25[a]
Chronic nephropathy	45	45	43	34	33	35	30	26

[a]Significantly different from control group ($p < 0.01$) according to χ^2 test.
[b]Significantly different from control group ($p < 0.05$) according to Fisher's exact test.
[c]Significantly different from control group ($p < 0.05$) according to χ^2 test.
[d]Significantly different from control group ($p < 0.01$) according to Fisher's exact test.

Source: Umeda et al. (2002).

In summary, the chronic toxicity and carcinogenicity study of male and female F344 rats administered biphenyl in the diet for 2 years (Umeda et al., 2002) provides evidence for biphenyl-induced bladder tumors in males, but not females, based on the development of transitional cell papillomas and carcinomas in the 4,500 ppm (378 mg/kg-day) males (Table 4-4). This study identified a no-observed-adverse-effect level (NOAEL) of 500 ppm (42.7 mg/kg-day) and a lowest-observed-adverse-effect level (LOAEL) of 1,500 ppm (128 mg/kg-day) for nonneoplastic kidney lesions (simple transitional cell hyperplasia in the renal pelvis and hemosiderin deposits) in female F344 rats exposed to biphenyl in the diet for 2 years. The chronic toxicity of biphenyl was assessed in Wistar rats (50/sex/group) administered the

chemical at 0, 0.25, or 0.5% (0, 2,500, or 5,000 ppm) in the diet for up to 75 weeks (Shiraiwa et al.,1989). The rats were observed daily for clinical signs. Body weight and food consumption were measured weekly. At death or scheduled sacrifice, gross pathologic examinations were performed and all organs were removed and preserved. Other than body weight and compound consumption data, the published results of this study were limited to kidney weight data and urolithiasis findings. Based on reported values for mean daily biphenyl intake (mg biphenyl/rat) and mean initial and final body weights for each study group, doses of biphenyl at the 0.25 and 0.5% dietary levels are estimated to have been 165 and 353 mg/kg-day for males, respectively, and 178 and 370 mg/kg-day for females, respectively.

Mean final body weights in both 2,500 and 5,000 ppm groups of biphenyl-exposed male and female rats were significantly lower (approximately 15 and 25% lower; $p < 0.01$) than their respective controls. Absolute and relative kidney weights of control and biphenyl-exposed rats were similar; with the exception of significantly increased ($p < 0.001$) mean relative kidney weight in 2,500 ppm female rats. The study authors reported the occurrence of hematuria (in both the 2,500 and 5,000 ppm groups) as early as week 16 and stated that it was more recognizable at 60 weeks (Shiraiwa et al., 1989). Kidney stone formation was reported in 6/46 and 1/43 of the 2,500 ppm males and females, respectively, and in 19/47 and 20/39 of the 5,000 ppm males and females, respectively. Detection of stones in other regions of the urinary tract was essentially limited to the 5,000 ppm groups and included the ureter (2/47 males and 2/39 females) and urinary bladder (13/47 males and 6/39 females). Kidney stones were hard, black, and located from the pelvic area to the medullary region. Stones in the ureter were hard, black, and composed of protein. Stones in the urinary bladder were hard, yellowish-white, round to oval in shape, and composed of ammonium magnesium phosphate. Histologically, kidneys with stones exhibited obstructive pyelonephritis accompanied by hemorrhage, lymphocytic infiltration, tubular atrophy, cystic changes of tubules, and fibrosis. Urinary bladders with stones exhibited simple or diffuse hyperplasia and papillomatosis of the mucosa; however, neoplastic lesions were not seen. No control rats (44 males and 43 females) showed stones in the kidney, ureter, or urinary bladder. The lowest exposure level in this study, 2,500 ppm in the diet for 75 weeks, was a LOAEL for formation of kidney stones associated with pyelonephritis in Wistar rats (dose levels of 165 and 178 mg/kg-day for males and females, respectively).

Shiraiwa et al. (1989) also reported the results of an initiation-promotion study in male Wistar rats (25/group) that included three groups administered a basal diet for 2 weeks followed by diets containing 0, 0.125, or 0.5% biphenyl (0, 1,250, or 5,000 ppm) for 34 weeks. Three other groups received diets containing 0.1% N-ethyl-N-hydroxyethylnitrosamine (EHEN, an initiator of kidney tumors in rats) for 2 weeks followed by diets containing 0, 0.125, or 0.5% biphenyl (0, 1,250, or 5,000 ppm) for 34 weeks. Initial and final body weights were recorded. At terminal sacrifice, gross pathologic examinations were performed. The study report included information regarding kidney weights, but did not indicate whether weights of other organs were

measured. Kidney and urinary bladder were fixed; kidneys were sectioned transversely (10–12 serial slices) and urinary bladders were cut into 4–6 serial slices. The authors used a computer-linked image analyzer to determine the incidence of kidney lesions and dysplastic foci. The presence of stones in the kidney and urinary bladder was assessed qualitatively using an infrared spectrophotometer. Based on reported values for mean daily biphenyl intake (mg biphenyl/rat) and average body weight (mean initial body weight + one-half the difference between mean initial and mean final body weight) for each study group, doses of biphenyl at the 0.125 and 0.5% dietary levels are estimated to have been 59.3 and 248.3 mg/kg-day, respectively, for rats on basal diet alone for the first 2 weeks and 62.0 and 248.2 mg/kg-day, respectively, for rats receiving EHEN in the diet for the first 2 weeks.

The mean final body weight of the rats receiving basal diet followed by diet containing 0.5% biphenyl was significantly lower ($p < 0.001$) than that of controls (0.389 ± 22 versus 0.432 ± 30 kg). It was stated that relative kidney weights were increased in this group of biphenyl-exposed rats compared to the basal diet control group, but the actual data were not presented. Stones were detected only in the rats receiving 0.5% biphenyl in the diet; incidences were 4/25 (kidney), 1/25 (ureter), and 3/25 (urinary bladder) in rats that had received that basal diet for the first 2 weeks. Similar results regarding final body weight and the detection of stones in the urinary tract were reported for the rats that had received EHEN in the diet prior to the administration of biphenyl. Incidences of dysplastic foci and renal cell tumors were determined in the kidneys of all groups of rats. Only rats that had received EHEN during the initial 2 weeks exhibited neoplastic kidney lesions (dysplastic foci, renal cell tumors). For the EHEN + 0% biphenyl, EHEN + 0.125% biphenyl, and EHEN + 0.5% biphenyl groups, incidences of rats with dysplastic foci were 25/25, 21/25, and 25/25, respectively, and incidences of rats with renal cell tumors were 13/25, 12/25, and 7/25, respectively. Under the conditions of this study, biphenyl did not exhibit tumor promoting characteristics for the kidney tumor initiator, EHEN.

Weanling albino rats (15/sex/group) were administered biphenyl in the diet at concentrations of 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, or 1% for 2 years (0, 10, 50, 100, 500, 1,000, 5,000, or 10,000 ppm) (Ambrose et al., 1960). Body weights were monitored every week during the period of active growth and then at 50-day intervals. Hemoglobin was monitored every 100 days in control and high-dose rats; at 500, 600, and 700 days in rats receiving 0.5% biphenyl, and at 500 and 600 days in rats receiving 0.1% dietary biphenyl. A 98-day paired-feeding experiment was conducted in which control rats were provided the same amount of food that rats of the 0.5 and 1.0% dietary biphenyl groups consumed to assess whether possible differences in growth would indicate a biphenyl exposure-related toxicological response or decreased palatability. At necropsy, the weights of liver, kidneys, heart, and testes were determined for all groups except those receiving 1.0% biphenyl in the diet. Stained sections of heart, lung, liver, kidney, adrenal, spleen, pancreas, stomach, intestine, bladder, thyroid, brain,

pituitary, and gonads were prepared for histopathologic examinations. In some cases, bone marrow smears were prepared.

The study report of Ambrose et al. (1960) did not include sufficient information from which daily biphenyl doses could be calculated. Biphenyl doses are estimated at 1, 4, 8, 42, 84, 420, and 840 mg/kg-day for the dietary levels of 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, and 1.0%, respectively, based on U.S. EPA (1988) reference values for body weight and food consumption in F344 rats (averages of values for males and females). There is greater uncertainty in the dose estimates at the two highest exposure levels because the magnitude of reported decreased food consumption in these groups was not specified in the study report. Decreased longevity was apparent in male and female rats of the 0.5 and 1.0% biphenyl exposure groups, but was not evident at lower exposure levels. Growth rates appeared similar among controls and groups exposed to biphenyl levels ≤0.1%. At the two highest exposure levels, markedly decreased growth was evident, but was attributable to decreased food consumption and indicative of decreased palatability based on results of the paired-feeding experiment. Decreased hemoglobin levels were reported in male and female rats of the two highest exposure levels after 300–400 and 500–600 days, respectively, but were considered at least partially related to lower food consumption in these groups relative to controls. Selected organ weights are summarized in Table 4-6. There were no statistically significant treatment-related effects on organ weights at dietary levels ≤0.1%, which were below those associated with decreases in food consumption, body weight, and survival (i.e., 0.5 and 1.0%). Relative liver and kidney weights of female rats of the 0.5% biphenyl exposure group were significantly ($p < 0.05$) increased, approximately 45 and 215% higher than those of respective controls. The only significant compound-related histopathological change occurred in the kidneys, which, in all members of the two highest exposure groups, showed irregular scarring, lymphocytic infiltration, tubular atrophy, and tubular dilation associated with cyst formation. Some evidence of hemorrhage was present, and calculi were frequently noted in the renal pelvis. Evidence of metaplasia in the epithelium of the renal pelvis did not implicate neoplastic activity, and, taking the histopathological results as a whole, there appeared to be no clear-cut, compound-related tumor development. However, the small number of animals in each group and the decreased survival in the two highest dose groups may have impaired the ability to detect late-developing tumors. The study identified 1,000 ppm biphenyl in the diet (84 mg/kg-day) as a NOAEL and 5,000 ppm (420 mg/kg-day) as the LOAEL for kidney effects including tubular atrophy and dilation associated with cyst formation and calculi formation in the renal pelvis of albino rats of both sexes.

Table 4-6. Body and organ weight data for male and female rats administered biphenyl in the diet for 2 years

Percent biphenyl in diet	Days on diets	Number of rats	Mean body weight (g) ± SE	Mean relative organ weight (g) ± SE			
				Liver	Kidneys	Heart	Testes
Males							
0.0	745	9	396 ± 24.6	2.89 ± 0.16	0.75 ± 0.02	0.32 ± 0.015	0.72 ± 0.03
0.001	744	8	424 ± 5.1	2.66 ± 0.06	0.70 ± 0.03	0.28 ± 0.008	0.62 ± 0.07
0.005	747	10	383 ± 19.8	2.84 ± 0.15	0.73 ± 0.02	0.30 ± 0.01	0.56 ± 0.06
0.01	752	11	394 ± 14.2	2.47 ± 0.07	0.72 ± 0.01	0.31 ± 0.008	0.67 ± 0.07
0.05	730	13	371 ± 15.8	3.03 ± 0.12	0.74 ± 0.02	0.31 ± 0.007	0.65 ± 0.06
0.1	746	10	366 ± 23.7	2.98 ± 0.19	0.83 ± 0.05	0.34 ± 0.012	0.60 ± 0.08
0.5	746	2	345	3.12	1.17	0.36	0.36
Females							
0.0	745	9	333 ± 9.4	3.11 ± 0.15	0.65 ± 0.01	0.33 ± 0.01	NA
0.001	744	6	369 ± 13.4	3.21 ± 0.17	0.62 ± 0.02	0.28 ± 0.07	NA
0.005	747	5	335 ± 16.6	2.81 ± 0.28	0.64 ± 0.02	0.31 ± 0.03	NA
0.01	752	11	341 ± 9.1	3.46 ± 0.74	0.62 ± 0.02	0.30 ± 0.01	NA
0.05	730	5	306 ± 12.5	3.51 ± 0.12	0.68 ± 0.02	0.31 ± 0.01	NA
0.1	746	5	327 ± 6.8	3.18 ± 0.10	0.65 ± 0.01	0.32 ± 0.01	NA
0.5	746	5	226 ± 25.8	4.52 ± 0.20[a]	1.39 ± 0.14[a]	0.46 ± 0.04	NA

[a]Significantly different from controls ($p < 0.05$) according to two-tailed Student's t-test.
NA = not applicable; SE = standard error of the mean

Source: Ambrose et al. (1960).

Male albino rats (8/group; strain not stated) were given biphenyl in the diet for up to 13 months at concentrations resulting in estimated doses of 250 or 450 mg/kg-day (Pecchiai and Saffiotti, 1957). Upon sacrifice, liver, kidney, spleen, heart, lung, thyroid, parathyroid, adrenal, pancreas, testis, stomach, and intestine were processed for histopathological examination. At 2-month interim sacrifices, moderate degenerative changes in liver and kidney were observed at both dose levels. Liver effects consisted of moderate degeneration and hypertrophy of the Kupffer cells with a generally well-preserved structure. Renal glomeruli were undamaged, but tubuli showed mild signs of degeneration. The liver and kidney effects did not appear to increase in severity in rats treated for up to 13 months. Other histopathologic effects noted in the biphenyl-treated rats included hypertrophied splenic reticular cells, small follicles with sparse colloid and desquamation of follicular epithelium in the thyroid, and hyperplastic and hyperkeratinized forestomach epithelium with occasional desquamation. Although the study report did not include tumor incidence data for the two dose groups, the study authors reported neoplastic lesions in the forestomach of three biphenyl-treated rats. Two of the rats exhibited papillomas of the forestomach epithelium (one after 7 weeks and one after 7 months of treatment); a squamous cell carcinoma was diagnosed in the other rat after 1 year of treatment.

The study authors noted two sequential responses to chronic biphenyl exposure: degenerative changes of nuclei and cytoplasm in the parenchyma of liver and kidney, spleen, thyroid, and adrenals within 2 months followed within 1 month or more by functional-regenerative changes that resulted in hyperplasia and nuclear hypertrophy of liver and kidney parenchyma as well as functional hyperactivity of the thyroid and parathyroid. Signs of cirrhosis were not seen, but irritation and hyperplasia were evident in the lower urinary tract. The lowest dose, 250 mg/kg-day biphenyl, was an apparent LOAEL for nonneoplastic degenerative changes in the liver, kidney, thyroid, and parathyroid of male albino rats resulting in hyperplasia of liver, kidney, and thyroid.

Sprague-Dawley rats (12/sex/group) were exposed to biphenyl in the diet for 2 years at exposure levels of 0, 0.01, 0.1, or 1% (0, 100, 1,000, or 10,000 ppm) (Dow Chemical Co., 1953). Body weights were monitored twice weekly for 3 months, then weekly. Blood samples were taken from all animals at the start of the experiment, approximately every 3 months thereafter, and at term. Hemoglobin levels, red and white blood cell counts and differential cell counts, and BUN concentrations were recorded. At death or scheduled necropsy, organ weights were recorded for liver, lung, kidneys, heart, and spleen. Sections from heart, liver, kidney, spleen, adrenals, pancreas, gonads, stomach, small and large intestine, voluntary muscle, lung, bladder, and brain were fixed and stained for histopathologic examination.

Based on U.S. EPA (1988) chronic reference values for body weight and food consumption in Sprague-Dawley rats (average values for combined sexes), doses of biphenyl for the dietary levels of 0.01, 0.1, and 1% are estimated to have been 7, 73, and 732 mg/kg-day, respectively. It is unclear to what extent the data in the study were compromised by an outbreak of pneumonia that affected the colony during the course of the experiment. Survival was poor in control males, all of which had died by 18 months. Only two of the females receiving 0.1% biphenyl in the diet survived to the end of the 21st month, and none had survived by the end of the 23rd month. However, the authors considered the decreased survival in this group of females to have been compound-related. Striking biphenyl concentration-related reductions in body weight gain were observed among the groups, although, in monitoring food efficiency, the authors indicated that the reduced growth was likely due to a lower daily consumption of food rather than to biphenyl toxicity. There were no clear indications of exposure-related changes in hematological parameters. The authors reported significant ($p < 0.05$) increases in average (combined sexes) relative liver and kidney weights at the highest exposure level, compared with control values (4.71 versus 3.05 g/100 g and 1.68 versus 1.00 g/100 g, respectively). Histopathologic examinations revealed dilatation of the kidney tubules, an effect that appeared to be associated with secondary inflammation, uremia, disruption of the filtration system, and an increase in BUN in affected animals. Tubular dilatation was evident in controls as well as treated animals, but increased in severity with dose (measured on a scale of 0-4). Among the controls, low-, mid-, and high-dose rats, respective incidences for tubular dilatation with severity

scores ≥2 were 1/12, 6/12, 7/12, and 11/12 for males and 1/12, 3/12, 4/12, and 11/12 for females. Incidences of tubular dilatation with severity scores ≥3 were 0/12, 1/12, 2/12, and 9/12 for males and 1/12, 2/12, 2/12, and 11/12 for females, respectively. Calcification and intratubular inflammation were frequently observed at the highest biphenyl exposure level. The incidence data for renal tubular dilatation with a severity score ≥3 (considered adverse) indicate that 100 ppm biphenyl in the diet (73 mg/kg-day) was a NOAEL and that 1,000 ppm (732 mg/kg-day) was a LOAEL for renal effects in Sprague-Dawley rats. The small number of rats in the exposure groups and the decreased survival at the highest exposure level may have impaired the ability to detect late-developing tumors in this study.

4.2.1.2.2. *Chronic mouse studies*

In a chronic toxicity and carcinogenicity study of BDF_1 mice (50/sex/group), biphenyl was administered in the diet for 2 years at concentrations of 0, 667, 2,000 or 6,000 ppm (Umeda et al., 2005). All animals were observed daily for clinical signs and mortality. Body weights and food consumption were recorded weekly for the first 14 weeks and every 4 weeks thereafter. Hematological and clinical chemistry parameters were measured in blood samples drawn from all 2-year survivors just prior to terminal sacrifice. At death or terminal sacrifice, gross pathological examinations were performed and organs were removed and weighed. Specific tissues prepared for microscopic examination were not listed in the study report, but included liver and kidney.

There were no overt clinical signs or effects on food consumption or survival among biphenyl-exposed mice of either sex compared to respective controls. However, mean terminal body weights showed a dose-related decrease; body weights were significantly less than those of respective controls at 2,000 and 6,000 ppm (males: 46.9, 43.1, 42.9, and 32.4 kg; females: 34.0, 32.5, 30.5, and 25.5 kg, at 0, 667, 2,000, and 6,000 ppm, respectively). Based on body weight and food consumption data, the study authors estimated that the 667, 2,000, and 6,000 ppm dietary levels resulted in average daily biphenyl doses of 97, 291, and 1,050 mg/kg-day in the males and 134, 414, and 1,420 mg/kg-day in the females.

Although there were no compound-related changes in hematological parameters, some clinical chemistry parameters showed marked changes in relation to dose, including a biphenyl dose-related increase in BUN that achieved statistical significance in 6,000 ppm males and females and 2,000 ppm males. In the female mice, dose-related increases in activities of the plasma enzymes AP, lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT; also referred to as AST), and glutamate pyruvate transaminase (GPT; also referred to as ALT) (see Table 4-7) suggested effects of biphenyl on the liver. Umeda et al. (2005) noted that females with malignant liver tumors exhibited extremely high AST, ALT, and LDH activities. In general, biphenyl did not induce dose-related changes in liver enzymes in male mice, although AP activity was significantly greater than controls in 6,000 ppm males (Table 4-7).

Table 4-7. Dose-related changes in selected clinical chemistry values from male and female BDF_1 mice exposed to biphenyl via the diet for 2 years

Males				
Biphenyl dietary concentration (ppm)	**0**	**667**	**2,000**	**6,000**
Dose (mg/kg-d)	**0**	**97**	**291**	**1,050**
Endpoint (mean ± SD)	**n = 34**	**n = 39**	**n = 37**	**n = 37**
AST (IU/L)	85 ± 92	58 ± 38	69 ± 60	88 ± 151
ALT (IU/L)	73 ± 113	34 ± 31	36 ± 49	43 ± 80
AP (IU/L)	178 ± 111	155 ± 30	169 ± 36	261 ± 102[a]
LDH (IU/L)	321 ± 230	252 ± 126	432 ± 868	283 ± 200
BUN (mg/dL)	20.2 ± 3.6	22.0 ± 4.0	23.2 ± 4.4[b]	22.9 ± 2.7[a]
Females				
Biphenyl dietary concentration (ppm)	**0**	**667**	**2,000**	**6,000**
Dose (mg/kg-d)	**0**	**134**	**414**	**1,420**
Endpoint (mean ± SD)	**n = 28**	**n = 20**	**n = 22**	**n = 31**
AST (IU/L)	75 ± 27	120 ± 110	211 ± 373[a]	325 ± 448[a]
ALT (IU/L)	32 ± 18	56 ± 46	134 ± 231[a]	206 ± 280[a]
AP (IU/L)	242 ± 90	256 ± 121	428 ± 499	556 ± 228[a]
LDH (IU/L)	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161[b]
BUN (mg/dL)	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7[a]

[a]Significantly different from controls ($p < 0.01$) according to Dunnett's test.
[b]Significantly different from controls ($p < 0.05$) according to Dunnett's test.

Source: Umeda et al. (2005).

The only apparent exposure-related effect on organ weights was 1.3-, 1.4-, and 1.6-fold increases in relative liver weights of 667, 2,000, and 6,000 ppm female mice, respectively (the data for liver weight group means and standard deviations [SDs] were not presented in Umeda et al. [2005]) Gross pathologic examinations revealed biphenyl dose-related increased incidences of liver nodules in females, but not males (Table 4-8). The nodules were round- or oval-shaped cystic or solid masses approximately 3–23 mm in diameter of the largest axis. Histopathological examinations revealed that 5, 16, and 19 of the nodule-bearing 667, 2,000, and 6,000 ppm female mice also exhibited proliferative lesions of hepatocellular origin (Table 4-8). Significantly increased incidences of basophilic cell foci were observed in 2,000 and 6,000 ppm female mice. Although incidences of basophilic cell foci were significantly increased in 667 ppm male mice as well, a dose-related effect was not evident because incidences of this lesion were not significantly increased in 2,000 or 6,000 ppm males compared to controls. Incidences of hepatocellular adenomas and combined incidences of hepatocellular adenomas or carcinomas were significantly increased in the 2,000 and 6,000 ppm females and Peto's trend tests confirmed

significant positive trends for dose-related increased incidences of hepatocellular adenomas ($p < 0.05$) and combined incidences of hepatocellular adenomas or carcinomas ($p < 0.01$). Incidences of hepatocellular carcinomas were significantly increased in 2,000 ppm females, but not 667 or 6,000 ppm females. However, Umeda et al. (2005) noted that the incidences of hepatocellular carcinomas (5/50 or 10%) in each of the 667 and 6,000 ppm groups of females exceeded the range of historical control data for that laboratory (26 hepatocellular carcinomas in 1,048 female mice [2.5% incidence in 21 bioassays, with a maximum incidence of 8%]). No significant biphenyl exposure-related effects on liver tumor incidences were seen in male mice. Incidences of desquamation of the urothelium in the renal pelvis were increased in 6,000 ppm male and female mice. Incidences of mineralization in the inner stripe of the outer medulla of the kidney were significantly increased in the 2,000 and 6,000 ppm female mice.

Table 4-8. Incidences of gross and histopathological findings in male and female BDF_1 mice fed diets containing biphenyl for 2 years

	Dietary concentration of biphenyl (ppm)							
	Males				Females			
	0	667	2,000	6,000	0	667	2,000	6,000
	Average dose (mg/kg-d)							
Parameter	0	97	291	1,050	0	134	414	1,420
Necropsy								
Liver nodules	20/50	16/49	14/50	11/50	7/50	13/50	24/50	26/49
Histopathology								
Liver[c]								
Adenoma	8/50	6/49	7/50	3/50	2/50	3/50	12/50[a]	10/49[a]
Carcinoma	8/50	8/49	5/50	4/50	1/50	5/50	7/50[a]	5/49
Adenoma or carcinoma (combined)	16/50	12/49	9/50	7/50	3/50	8/50	16/50[b]	14/49[a]
Basophilic cell foci	0/50	6/49[b]	1/50	2/50	1/50	1/50	12/50[b]	6/49[a]
Clear cell foci	0/50	6/49[b]	2/50	0/50	2/50	1/50	3/50	2/49
Eosinophilic cell foci	0/50	0/49	0/50	0/50	0/50	1/50	0/50	0/49
Kidney								
Desquamation: pelvis	0/50	0/49	0/50	10/50[b]	4/50	0/50	0/50	15/49[b]
Mineralization inner stripe–outer medulla	9/50	8/49	14/50	14/50	3/50	5/50	12/50[a]	26/49[b]

[a]Significantly different from controls ($p < 0.05$) according to Fisher's exact test.
[b]Significantly different from controls ($p < 0.01$) according to Fisher's exact test.
[c]Historical control data for hepatocellular tumors: Male BDF_1 mouse: adenoma—17.2% (4–34%), carcinoma—18.8% (2–42%), adenoma/carcinoma—32.2% (10–68%). Female BDF_1 mouse: adenoma—4.8% (0–10%), carcinoma—2.3% (0–8%), adenoma/carcinoma—7.1% (2–14%). Source: email dated July 25, 2011, from Umi Umeda, JBRC, to Connie Kang, NCEA, ORD, U.S. EPA.

Source: Umeda et al. (2005).

In summary, the chronic toxicity and carcinogenicity study of male and female BDF_1 mice administered biphenyl in the diet for 2 years (Umeda et al., 2005) provides evidence for

biphenyl-induced liver tumors in females, but not males, based on significantly increased incidences of hepatocellular adenomas and combined carcinomas or adenomas in the female mice receiving biphenyl from the diet at 414 and 1,420 mg/kg-day (Table 4-8). This study identified a NOAEL of 134 mg/kg-day and a LOAEL of 414 mg/kg-day for nonneoplastic effects (mineralization in the kidney and significantly increased plasma ALT and AST activities) in female BDF_1 mice exposed to biphenyl in the diet for 2 years.

Groups of female ddY mice were fed diets containing 0 (n = 37 mice) or 0.5% (n = 34 mice) biphenyl (5,000 ppm) in the diet for 2 years (Imai et al., 1983). This study also included groups exposed to dietary concentrations of 0.2% thiabendazole or a mixture of 0.25% biphenyl and 0.1% thiabendazole (results from this part of the study are not further described herein). Food consumption, body weights, and survival were assessed at intervals throughout exposure. At terminal sacrifice, several organs were weighed. The following organs were examined for histopathological changes: brain, pituitary, thymus, liver, spleen, pancreas, lung, heart, adrenal, kidney, ovaries, uterus, thyroid, stomach, small intestine, and large intestine. Urine and blood samples were collected from mice (6-12/group) at terminal sacrifice and were analyzed for urinalysis, hematological, and serum chemistry endpoints. Based on U.S. EPA (1988) methodology for estimating food consumption rates from body weight data and the reported average terminal body weight for the 5,000 ppm mice (0.037 kg), an oral dose of 855 mg/kg-day is estimated from the dietary exposure.

Exposure to biphenyl did not influence survival, food consumption, or growth compared with controls. No marked exposure-related effects were found on terminal organ and body weights or on the urinalytic, hematologic, or serum chemistry endpoints. Histological examination revealed no increased incidence of non-neoplastic lesions in examined tissues in the 5,000 ppm biphenyl group, compared with the control group. The only tissues showing tumors at elevated incidence in the 5,000 ppm mice, compared with the control group, were the lung (11/34 [32.4%] versus 9/37 [24.3%] in controls) and lymphatic tissues (lymphomas: 5/34 [14.7%] versus 4/37 [10.8%]; leukemia: 3/34 [8.8%] versus 2/37 [5.4%]), but these increases were not statistically significant ($p > 0.05$ by the Fisher's exact test). In summary, 5,000 ppm biphenyl in the diet of female ddY mice for 2 years was a NOAEL for non-neoplastic lesions, survival, body and organ weight changes, and changes in urinalytic, hematologic, and serum chemistry endpoints. No carcinogenic response occurred in female ddY mice exposed to 5,000 ppm biphenyl in the diet (estimated dose of 855 mg/kg-day) for 2 years (Imai et al., 1983).

The carcinogenic potentials of 130 chemicals, including biphenyl, were assessed in a protocol that exposed groups of two strains of F1 hybrid mice (18/sex/strain/group), produced by mating female C57BL/6 mice to either male C3H/Anf mice (F1 designated as strain A) or male AKR mice (F1 designated as strain B) to individual chemicals by the oral route for 18 months (Innes et al., 1969; NCI, 1968). Four groups of untreated controls and a group of gelatin vehicle controls (18/sex/strain/group) were included in the study. In the case of biphenyl, the chemical

was administered via gavage to mice for 3 weeks, starting at the age of 7 days at 215 mg biphenyl/kg body weight in 0.5% gelatin (the report of Innes et al. (1969) appears to have erroneously reported the gavage dose as 2.5 mg/kg). Thereafter, and for the rest of the experimental period, biphenyl was mixed with chow to a final concentration of 517 ppm. The gavage dose level and food concentration of biphenyl were selected to reflect the maximum tolerated dose identified in preliminary range-finding, single-dose subcutaneous injection and single- and repeated-dose oral administration studies. Initial gavage dose and dietary levels of biphenyl were not adjusted for weight gain during the 18-month study. Based on U.S. EPA (1988) chronic reference values for body weight and food consumption in strain A mice (average values for combined sexes), an average oral dose of 91 mg/kg-day is estimated from the dietary exposure. Blood smears were prepared from mice that showed splenomegaly, liver enlargement, or lymph adenopathy at necropsy. At term, mice were examined for any gross pathological features. Major organs were processed for histopathologic examination (including total chest contents, liver, spleen, kidneys with adrenals, stomach, and genital organs). Innes et al. (1969) reported incidences for hepatomas, pulmonary tumors, and lymphomas in control mice and for tested chemicals that were judged to give "high tumor yield"; biphenyl was reported to be noncarcinogenic, but tumor incidence data for biphenyl were not reported. The NCI (1968) report included tabulated incidences of hepatomas, pulmonary tumors, and lymphomas in control mice and biphenyl-treated mice, which are summarized in Table 4-9. In summary, the results provide no evidence of a carcinogenic response to 18 months of oral exposure to biphenyl in mice (215 mg/kg by gavage for 3 weeks, followed by dietary exposure to 517 ppm biphenyl).

Table 4-9. Incidences of selected tumor types among controls and mice administered biphenyl orally for 18 months

Group	Incidences of selected tumor types[a]		
	Hepatoma	Pulmonary tumors	Reticular cell sarcoma
Strain A male mice			
Controls	8/79	5/79	5/79
Biphenyl-treated	2/17	3/17	1/17
Strain A female mice			
Controls	0/87	3/87	4/87
Biphenyl-treated	0/18	1/18	0/18
Strain B male mice			
Controls	5/90	10/90	1/90
Biphenyl-treated	3/17	1/17	0/17
Strain B female mice			
Controls	1/82	3/82	4/82
Biphenyl-treated	0/17	0/17	4/17

[a]Tumor incidences were tallied from those mice for which histopathologic examinations were performed.

Source: NCI (1968).

4.2.1.2.3. *Chronic studies in other animal species*

Mongrel dogs (two males and one female/group) were administered 0, 2.5, or 25 mg/kg biphenyl in corn oil by capsule 5 days/week for 1 year (Monsanto, 1946). Dogs were examined daily for clinical signs and weighed weekly. Blood samples were drawn at 3-month intervals to measure hematological and clinical chemistry parameters. Urine samples were obtained at similar intervals to measure specific gravity, sugar, protein, bile pigments, occult blood, and microscopic sediment. Samples of urine from the high-dose dogs were collected during week 18, pooled, and analyzed for the presence of biphenyl and metabolites. At termination, gross necropsies were performed, and sections of large and small intestine, pancreas, ovary or testis, adrenal, urinary bladder, stomach, lung, thyroid, brain, heart, spleen, and liver were prepared for histopathologic examination. Although slight fluctuations were seen in body weight during the study, the dogs generally exhibited a net weight gain. Fluctuations in hematological parameters and urine analysis were inconsistent and not considered compound-related. Gross pathological examination of the dogs showed no obviously compound-related effects. Histopathologic examinations revealed lung congestion consistent with bronchial pneumonia in one high-dose dog; histopathology was unremarkable for each of the other dogs in the study.

Dow Chemical Co. (1953) described a biphenyl feeding experiment in which four groups of Rhesus monkeys (two males and one female/group) were exposed to 0, 0.01, 0.1, or 1% biphenyl in chow for 1 year, during which time most of the animals experienced ill health not related to biphenyl exposure. Despite this caveat, hematological parameters were normal. The

authors considered an increase in relative liver weight in high-dose monkeys (4.65 g/100 g body weight versus 3.90 g/100 g body weight in controls) to possibly be compound-related.

4.2.2. Inhalation Studies

In three separate experiments, albino rabbits (sex and strain not stated), Sprague-Dawley rats (sex not stated), and mice (sex and strain not stated) were repeatedly exposed to dusts composed of 50% biphenyl attached to celite for 7 hours/day, 5 days/week (Deichmann et al., 1947; Monsanto,1946). In the first experiment, 3 rabbits and 10 rats were exposed to an average concentration of 300 mg/m^3 on each of 64 days over a period of 94 days. The rats exhibited irritation of the nasal mucosa accompanied by serosanguineous discharge. Five of the rats died prior to term, and the survivors lost weight. The rabbits exhibited no exposure-related adverse signs. In the second experiment, three rabbits and six rats were exposed to an average concentration of 40 mg/m^3 on each of 46 days over a total period of 68 days. One rat died prior to term. The surviving rats showed signs of mucous membrane irritation, but appeared to gain weight at a normal rate. The rabbits exhibited no exposure-related adverse signs. In the third experiment, 12 mice and 4 rats were exposed to an average concentration of 5 mg/m^3 on each of 62 days over a total period of 92 days. While the rats were unaffected at this concentration, all of the mice showed signs of irritation of the upper respiratory tract and two died prior to term. Bronchopulmonary lesions (including acute emphysema, congestion, edema, bronchitis, widespread lobular pneumonia, and multiple pulmonary abscesses) were reported in rats from experiments 1 and 2 and in mice of experiment 3. Some unspecified minor liver and kidney lesions were also noted. Based on the results of these three experiments, a LOAEL of 5 mg/m^3 in mice and a LOAEL of 40 mg/m^3 in rats for upper respiratory tract irritation was identified.

Groups of CD-1 mice (50/sex/group) were exposed to airborne biphenyl at vapor concentrations of 0, 25, or 50 ppm (0, 157.7, and 315.3 mg/m^3, respectively) for 7 hours/day, 5 days/week for 13 weeks (Sun Company Inc., 1977a). Mice were maintained and exposed to biphenyl in groups of 5 (for a total of 10 groups/sex/exposure group). All animals were checked daily for clinical signs and mortality, and body weight data were collected. Upon completion of the 13-week exposure period, surviving mice were placed in metabolic cages for 12-hour collection of urine for urinalysis. Blood samples were collected for blood chemistry and hematology assessments. Gross and histopathologic examinations were performed on all mice. Ten surviving mice/sex/group were held for a 30-day recovery period prior to terminal sacrifice.

During the first few days of biphenyl exposure, some of the test material crystallized in the delivery system; analysis of biphenyl exposure levels was not performed on these days. Daily measured biphenyl exposure concentrations were highly variable during the first half of the 13-week exposure period, whereas subsequently measured concentrations were closer to target concentrations. For example, during the first 45 exposure sessions, measured daily biphenyl concentrations in the 50 ppm target groups ranged from as low as 5 ppm to as high as

102 ppm and subsequent measurements ranged from 48 to 55 ppm. Mean biphenyl concentrations (± 1 SD) calculated for the entire 13 weeks of exposure were 25 ± 7 and 50 ± 16 ppm for the 25 and 50 ppm target groups, respectively. The authors reported the loss of 46 mice (40 males and 1 female at 25 ppm and 5 males at 50 ppm) due to overheating and cannibalization. Since the overheating event occurred after 46 exposures, the overall study duration ran for 117 days to ensure that replacement mice received a total of 65 exposures as called for in the protocol. The study report did not mention results of clinical observations, and mortality data were not specifically summarized. There were no clear indications of exposure-related effects on body weights. Results of urinalysis, hematology, and clinical chemistry did not indicate any clear exposure-related changes that could be attributed to biphenyl toxicity. Gross and histopathological examinations revealed congested and hemorrhagic lungs, hyperplasia of the trachea with inflammation accompanied by a high incidence of pneumonia, and congestion and edema in liver and kidney of biphenyl-exposed mice (Table 4-10). The pathologist considered the congestion in the lung, liver, and kidney a likely effect of the anesthetic used for killing the mice, although control mice did not exhibit these effects at 13-week sacrifice. The hemorrhagic lungs and tracheal hyperplasia were considered effects of biphenyl exposure. Results from the 30-day recovery groups suggest that the biphenyl exposure-related pulmonary effects were reversible. This study identified a LOAEL of 25 ppm for histopathologic lung, liver, and kidney lesions in male and female CD-1 mice exposed to biphenyl by inhalation for 7 hours/day, 5 days/week for 13 weeks.

Table 4-10. Incidences of selected histopathologic lesions in tissues of CD-1 mice exposed to biphenyl vapors 7 hours/day, 5 days/week for 13 weeks

Effect	13-Week exposure groups[a]		
	0 ppm	25 ppm	50 ppm
Pulmonary congestion, edema	0/80	95/98	71/71
Pneumonia	0/80	15/98	20/71
Tracheal hyperplasia	0/80	80/98	70/71
Hepatic congestion, edema	0/80	87/98	71/71
Renal congestion, edema	0/80	87/98	71/71

[a]The study report presented incidences of histopathologic lesions for combined male and female mice only; no statistical analyses were conducted.

Source: Sun Company Inc. (1977a).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

Pregnant female Wistar rats (18–20 group) were gavaged with 0, 125, 250, 500, or 1,000 mg/kg-day biphenyl in corn oil on gestation days (GDs) 6–15 (Khera et al., 1979). Body

weights of dams were recorded on GDs 1, 6–15, and 22, at which point all dams were sacrificed. Parameters evaluated at autopsy included the number of corpora lutea, fetal weights and viability, and early resorptions. Two-thirds of the live fetuses/litter were examined for skeletal development and the rest were examined for the presence of visceral abnormalities.

Five of the 20 high-dose dams died prior to sacrifice. Doses ≤500 mg/kg-day produced no clinical signs of maternal toxicity or evidence of treatment-related effects on maternal weight gain. As shown in Table 4-11, a significantly increased number of dams without live fetuses was observed in the high-dose group, compared with controls. Mean numbers of corpora lutea and live fetuses in the high-dose dams were similar to those of controls and dams of all other dose levels. However, the percent of dead fetuses and resorption sites was higher in the high-dose group, and the numbers of anomalous fetuses and litters bearing anomalous fetuses appeared to increase with increasing dose. The increases in the number of fetuses with anomalies, such as missing and unossified sternebrae or delayed calvarial ossification, were not statistically significant, but, as shown in Table 4-11, the incidence of litters with any type of fetal anomalies ("anomalous litters/number examined") was statistically significantly elevated ($p < 0.05$ by Fisher's exact test) at ≥500 mg/kg-day compared with control incidences. This study identified a NOAEL of 500 mg/kg-day and a LOAEL of 1,000 mg/kg-day for frank maternal toxicity (increased mortality and decreased dams with live fetuses) and lethal fetal effects. For less severe developmentally toxic effects (increased incidence of anomalous litters), 500 mg/kg-day was a LOAEL and 250 mg/kg-day was a NOAEL.

Table 4-11. Prenatal effects following oral administration of biphenyl to pregnant Wistar rats on GDs 6–15

Effect	Dose (mg/kg-d)				
	0	125	250	500	1,000
Rats without live fetuses at term/number mated	2/18	0/20	1/19	2/20	11/20[a]
Corpora lutea/pregnancy (mean ± SE)	12.6 ± 0.4	12.9 ± 0.4	13.7 ± 0.5	13.3 ± 0.4	12.5 ± 0.7
Live fetuses/pregnancy (mean ± SE)	11.3 ± 0.7	11.8 ± 0.6	11.9 ± 0.6	11.2 ± 0.5	10.7 ± 1.3
Dead or resorbed fetuses (%)	4.8	3.3	6.1	7.8	13.7[b]
Fetal weight (g mean ± SE)	5.1 ± 0.1	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	4.5 ± 0.3
Anomalous fetuses/number examined	17/176	22/236	22/213	35/199[c]	25/107[c]
Anomalous litters/number examined	8/16	11/20	13/18	15/18[c]	6/9
Anomalies (number of fetuses affected)					
Wavy ribs, uni- and bilateral	3	7	9	8	5
Extra ribs, uni- and bilateral	9	12	9	15	6
13th rib, small sized	1	1	2	1	0
Sternebrae, missing or unossified	4	3	4	16	17
Calvarium, delayed ossification	0	2	0	0	8
Miscellaneous	1	1	1	0	0

[a]Significantly ($p < 0.05$) different from control incidence according to Fisher's exact test. Five dams died prior to scheduled sacrifice, five other dams were not pregnant at term, and one dam had seven resorption sites and no live fetuses.
[b]Derived from nine pregnant dams with live fetuses and one dam with seven resorptions and no live fetuses. The study author stated that the percentage of dead or resorbed fetuses in the 1,000 mg/kg dose group was not statistically significantly different from controls.
[c]Significantly ($p < 0.05$) different from controls according to Fisher's exact test.

Source: Khera et al. (1979).

Dow Chemical Co. (1953) reported the results of a multigenerational study in which groups of 4-month-old male and female Long Evans rats (three males and nine females/group) were fed diets containing 0, 0.01, 0.1, or 1.0% biphenyl. Based on U.S. EPA (1988) subchronic reference values for body weight and food consumption in male and female Long Evans rats, doses of biphenyl for the dietary levels of 0.01, 0.1, and 1.0% are estimated to have been 9, 89, and 887 mg/kg-day, respectively, for the males and 10, 101, and 1,006 mg/kg-day, respectively, for the females. Average cross-gender doses for males and females were 10, 95, and 947 mg/kg-day. For breeding, three females were placed together with one male. Following the breeding phase, females were separated and number of litters cast, number of days between mating and delivery, and average number of pups/litter at delivery were recorded. F1 pups were weighed and culled to seven/litter at 2 days of age and weaned at 3 weeks of age, and weights were recorded weekly for postnatal weeks 3–6. The F1 rats were continued on the same diets as their parents, and, at 10 weeks of age, nine F1 females and three F1 males were mated to produce an F2 generation of pups. F2 pups were selected (by the same procedure) for mating and

production of an F3 generation that were sacrificed at 3 weeks of age; 12 F3 pups from each diet group were subjected to gross pathologic examinations.

There were no significant differences between controls and 0.01 and 0.1% biphenyl-fed groups regarding litters cast, gestation length, or average number or weight of pups/litter at birth or at 3 or 6 weeks of age. Decreased fertility in the 1% biphenyl-fed group of females was observed (6/9, 7/9, and 8/9 confirmed pregnancies for the three successive generations of 1.0% biphenyl-fed groups versus 8/9, 9/9, and 8/9 confirmed pregnancies for controls). Averaged for F1, F2, and F3 pups combined, the 1.0% biphenyl-fed group exhibited significantly ($p < 0.05$) decreased number of pups/litter at birth (6.2/litter versus 8.6/litter for controls) and lower average body weight at 3 weeks of age (36 versus 48 g for controls) and 6 weeks of age (78 versus 113 g for controls). Gross pathologic evaluations of F3 weanlings revealed no signs of biphenyl treatment-related effects. There was no evidence of a cumulative effect over the three generations. The study authors indicated that the decreased fertility, smaller litter size, and reduced rate of growth in the 1.0% biphenyl-fed group may have been associated with unpalatability and resultant decreased food intake.

The research report of Ambrose et al. (1960) contains a subsection in which the reproductive toxicity of biphenyl was examined in two experimental series. In the first experiment, weanling albino rats were administered 0 or 0.1% biphenyl (5 males and 10 females/group) or 0.5% biphenyl (3 males and 9 females) in the diet for 60 days prior to mating. In the second experiment, groups of 90-day-old albino rats were administered 0 or 0.1% biphenyl (4 males and 8 females/group) or 0.5% biphenyl (3 males and 9 females) in the diet for 11 days prior to mating. Based on U.S. EPA (1988) subchronic reference values for body weight and food consumption in rats of unspecified strain (average values for combined sexes), doses of biphenyl for the dietary levels of 0.1 and 0.5% are estimated to have been 105 and 525 mg/kg-day, respectively. All rats were maintained on their respective diets throughout mating and until the progeny of all litters were weaned. Insufficient information is provided in the report to permit a judgment as to whether dietary exposure to biphenyl was associated with reproductive deficits. However, the authors presented tabular data for number of rats casting litters, total born, and range of litter size (Table 4-12) and concluded that the compound had no significant effect on reproduction.

Table 4-12. Summary of reproductive data in albino rats exposed to dietary biphenyl

Experimental series	Diet[c]	Dams with litters	Total offspring	Litter size (range)
First[a]	Control	9/10	59	3–9
	0.1% biphenyl	10/10	67	2–10
	0.5% biphenyl	8/9	53	3–9
Second[b]	Control	8/8	64	5–13
	0.1% biphenyl	6/8	63	3–10
	0.5% biphenyl	8/9	48	3–9

[a]Weanling rats on diets for 60 days before mating.
[b]90-Day-old rats on diets for 11 days before mating.
[c]0.1% = 105 mg/kg and 0.5% = 525 mg/kg/day

Source: Ambrose et al. (1960).

4.3.2. Inhalation Exposure

No studies were identified that examined the reproductive/developmental toxicity of biphenyl via the inhalation route.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute and Short-term Toxicity Data

Acute oral toxicity studies of biphenyl provide median lethal dose (LD_{50}) values ranging from 2,180 to 5,040 mg/kg for rats (Pecchiai and Saffiotti, 1957; Union Carbide, 1949; Deichmann et al., 1947; Monsanto, 1946) and an LD_{50} value of 2,410 mg/kg for rabbits (Deichmann et al., 1947). Dow Chemical Co. (1939) reported 100% survival and 100% lethal doses of 1,600 and 3,000 mg/kg, respectively, in rats. Clinical signs commonly observed following single oral dosing in these studies included increased respiration, lacrimation, loss of appetite and body weight, and muscular weakness. Deaths occurred in the first few days following dosing. Typical targets of histopathologic lesions were lungs, liver, and upper gastrointestinal tract.

Groups of mice (10/sex of unspecified strain) were exposed to biphenyl by inhalation for 4 hours at average analytical concentrations of 14.11, 38.40, or 42.80 ppm (89.0, 242.2, and 270.0 mg/m^3, respectively) and observed for up to 14 days following exposure (Sun Company Inc., 1977a, b). Clinical signs of hyperactivity and mild respiratory discomfort were noted during exposure, but resolved during postexposure observation. One male mouse of the 42.80 ppm group died after 2 hours of exposure, but this death was not attributed to biphenyl exposure. All other mice survived throughout the 14-day postexposure observation period. Slight lung congestion was noted in most mice upon gross pathological examination.

Sun Company Inc. (1977b) also provided details of a study in which groups of mice (10/sex of unspecified strain) were exposed to biphenyl for 7 hours/day, 5 days/week for 2 weeks at average analytical concentrations of 0, 24.8, or 54.75 ppm (0, 156.4, and 345.5 mg/m^3, respectively). Five animals/group were sacrificed immediately after exposure; the remaining animals were sacrificed following a 14-day recovery period. Clinical signs were monitored daily. Gross pathologic examinations at necropsy included assessment of lungs, trachea, heart, spleen, liver, kidneys, stomach, and intestines. Histopathologic examinations included tissues from lung, trachea, kidney, spleen, and liver. The study authors reported signs of hyperactivity in some mice during the first few exposure periods. One female mouse of the 24.8 ppm exposure group died prior to the third exposure session and one control female mouse died prior the final exposure session. No abnormal clinical signs were seen during the 14-day recovery period. Gross and histopathologic examinations revealed no signs of exposure-related adverse effects.

Four rabbits (sex and strain unspecified) received up to 20 daily doses of 500 mg/kg "purified" biphenyl to the skin; the compound was applied as a 25% preparation in olive oil. Three rabbits received the same concentration of technical biphenyl (Deichmann et al., 1947; Monsanto, 1946). The compound was left on the skin for 2 hours and then washed off with soap and water. Some biphenyl derivatives were similarly assessed. One rabbit receiving purified biphenyl died after eight applications, and the rest of the animals survived to term. The only reported sublethal effect clearly associated with biphenyl exposure was that of weight loss, averaging 45 and 172 g for the rabbits receiving purified and technical biphenyl, respectively.

4.4.2. Kidney/Urinary Tract Endpoint Studies

Endpoint-specific studies of biphenyl-induced urinary tract effects in rats (Shibata et al., 1989b; Shibata et al., 1989a; Kluwe, 1982; Søndergaard and Blom, 1979; Booth et al., 1961) support findings of the chronic oral rat studies described in Section 4.2.1.2 (Chronic Toxicity and Carcinogenicity). Detailed descriptions of these endpoint-specific studies are presented below.

In a preliminary study, five adult rats (sex and strain unspecified) were administered biphenyl in the diet at 1% (w/w) for 26 days followed by a 29-day postexposure recovery period for a total study period of 55 days (Booth et al., 1961). Total urine volume and the volume of sulfosalicylic acid-precipitable sediment were recorded from urine collected from all five rats on study days 4, 8, 18, 20, and 26 (exposure days), and study days 28, 32, 35, and 54 (recovery period). Volumes of both urine and sulfosalicylic acid-precipitable sediment increased from 7 and 0.56 mL, respectively, on exposure day 4 to 32 and 2.24 mL, respectively, on exposure day 20. Both values remained relatively high (approximately 27 and 2.2 mL, respectively) on exposure day 26 and decreased to approximately 14 and 0.8 mL, respectively, by the end of the recovery period. Fractionation and analysis of the precipitate suggested the presence of p-hydroxybiphenyl and its glucuronide. The study authors indicated that similar effects were noted

in male and female rats receiving biphenyl at a level of 0.5% in the diet, but not at the 0.1% dietary level.

A follow-up study employed 42 rats/sex/group and biphenyl dietary levels of 0, 0.1, 0.25, or 0.5% (w/w). Biphenyl doses are estimated at 83.7, 209, and 419 mg/kg-day for the dietary levels of 0.1, 0.25, and 0.5%, respectively, based on U.S. EPA (1988) chronic reference values for body weight and food consumption in F344 rats (averages of values for males and females). Rats were exposed for up to 165 days and followed for 0, 30, or 60 days of recovery. Urine samples were collected periodically from five rats/sex/exposure group. Interim sacrifices of five rats/sex/exposure group were performed after 30, 60, and 120 days on the diet in order to assess the progression of biphenyl-induced histopathological effects on the kidney. As noted in the preliminary study, the rats of the 0.5% exposure group in the follow-up study exhibited gradual increases in the urine volume and sulfosalicylic acid-precipitable sediment and decreased in both parameters during postexposure recovery. The study authors indicated that these effects were much less pronounced in the 0.25% exposure group and absent in the 0.1% exposure group. At the 0.5% exposure level, kidney lesions were noted in 1/5 of the males (several small cysts and dilated tubules in the medulla and inner cortex) and 2/5 of the females (mild local tubular dilation with some epithelial flattening) following 30 days of exposure. Similar, but more extensive, kidney lesions were noted in 3/5 males and 5/5 females following 60 days of exposure. The kidney lesions were even more prominent following 120 days of exposure. Reported histopathologic findings in the kidneys of rats from the 0.25% exposure group were limited to a single instance of an unspecified "prominent kidney lesion" at 60 days, and one small calculus in the pelvis of one rat and a small calcareous deposit in the renal pyramid of another rat following 120 days of exposure. Urinary and histopathologic renal effects were not assessed at the end of the 165-day treatment period; however, during the 60-day postexposure recovery period, rats of the 0.5% biphenyl exposure group exhibited a regression of kidney lesions and improvement in urine quality.

Kluwe (1982) examined changes in urine composition and kidney morphology in F344 rats exposed to biphenyl. Groups of male F344 rats were administered biphenyl (in corn oil) by single gavage dosing at 0, 250, 500, or 1,000 mg/kg and observed for 15 days following treatment. Body weights were recorded, and urine was collected on days 1, 2, 3, 4, 8, and 15 following treatment for urinalysis. Interim sacrifices were performed on eight control and eight high-dose rats on posttreatment days 1, 2, 3, 8, and 15 for assessment of weight and histopathology of the kidney. There were no significant effects on body weight in the low-dose group. Mean body weight gains of mid- and high-dose groups were consistently 6–10% lower than control values ($p < 0.05$), beginning as early as day 2 following the initiation of dosing and continuing through day 15. Dose-related increases in polyuria, proteinuria, and glucosuria were observed on day 1; polyuria and glucosuria were no longer apparent by day 4 and proteinuria resolved between days 8 and 15. Histopathologic examinations of kidneys revealed renal

papillary necrosis in 8/32 high-dose rats; this effect was observed as early as day 1 and persisted during the 15-day posttreatment period.

Kluwe et al. (1982) conducted a similar experiment in which groups of male F344 rats received biphenyl at doses of 0, 250, or 500 mg/kg-day by gavage for 14 days. In this experiment, polyuria persisted throughout the treatment period; glucosuria was no longer apparent by day 4 and proteinuria resolved between treatment days 8 and 15. Relative kidney weight of high-dose rats was significantly increased during the second half of the treatment period, but the magnitude of this effect was small and considered by the study authors to be of little biological significance. There was some indication of tubular dilatation in focal areas of kidneys from the high-dose rats.

Groups of male and female SPF-Wistar rats were administered diets consisting of semisynthetic chow and biphenyl at concentrations resulting in biphenyl doses of 0, 50, 150, 300, or 450 mg/kg-day (Søndergaard and Blom, 1979). Other groups were administered diets consisting of commercial chow and biphenyl at concentrations resulting in biphenyl doses of 0, 50, 150, 300, 500, or 1,000 mg/kg-day. The treatment period lasted for up to 21 days. The numbers of male and female rats in each treatment group are specified in Table 4-13. Urine was collected on days 4, 10, and 17 for urinalysis. At terminal sacrifice, absolute and relative kidney weights were determined and kidney tissues were prepared for light and electron microscopic assessment. Apparently, interim sacrifices (days 1, 2, 4, and 10) were performed in order to assess the activity of AP in proximal tubules. Table 4-13 presents semiquantitative study results, which include increases in urine volume/specific gravity and relative kidney weight, as well as polycystic kidney changes. No changes in AP levels were seen as a result of biphenyl exposure. The kidney effects of biphenyl appeared to be more pronounced when added to the semisynthetic diet versus the commercial diet, with 50 mg/kg-day as a LOAEL for the onset of kidney changes.

Table 4-13. Number of Wistar rats exposed to biphenyl and the degree of change in kidney weight and cellular architecture

Exposure (mg/kg-d)	Number of animals (male/female)	Relative kidney weight increases	Cystic change	Increases of urine volume/specific gravity
Semisynthetic diet				
0	3/14	–	–	–/–
50	4/3	+	–	
150	0/10	+	*	●/●
300	14/14	+++	***	
450	4/4	+++	***	
Commercial chow				
0	10/20	–	–	–/–
50	10/10	–	–	
150	10/10	–	–	
300	10/10	–	–	
500[a]	0/10	+[b]	–	●/●
1,000[a]	0/10	+++[b]	**	●/●

[a]Dose for 14 days.
[b]Absolute organ weight.

+ = statistically significant compared with controls ($p < 0.05$), as calculated by the authors (Student's t-test);
+++ = statistically significant compared with controls ($p < 0.001$), as calculated by the authors (Student's t-test);
* = less than one-third of the area; ** = less than two-thirds of the area; *** = greater than two-thirds of the area;
● = effect; – = no effect

Source: Søndergaard and Blom (1979).

Male F344 rats (20/group) were exposed to 0 or 0.5% (w/w) biphenyl in the diet for 24 weeks (Shibata et al., 1989a). After 4 weeks, 5 rats/group were injected with 100 mg/kg 5-bromo-2-deoxyuridine (BrdU) and sacrificed 1 hour later. One kidney from each rat was processed for immune-histopathologic identification of BrdU as an index of cell proliferation, while the second kidney was processed for light and scanning electron microscopic examination. The remaining rats were sacrificed after 8, 16, and 24 weeks to monitor further development of morphological alterations in the renal papilla and pelvis. Survival was unaffected by treatment and biphenyl-treated animals showed no adverse clinical signs. The study authors reported that treatment resulted in significantly lower mean body weight compared to controls; food consumption was unaffected and water consumption was slightly higher than that of controls. There were no significant treatment-related effects on labeling indices of cell proliferation (BrdU incorporation) in renal papilla or pelvic epithelia, and no histopathologic lesions of the renal papilla and pelvis were evident. Focal calcification of the renal medulla was observed in the majority of the biphenyl-treated rats. The study authors stated that urinalysis demonstrated an association between biphenyl exposure and microcalculi formation, but provided no additional information regarding urinalysis results.

In a similar study (Shibata et al., 1989b), a group of 10 male F344 rats received 0.5% (w/w) biphenyl in the diet for up to 8 weeks. Based on U.S. EPA (1988) subchronic reference values for body weight and food consumption in male F344 rats, the dose was estimated at 500 mg/kg-day. At 4 weeks, five rats/group were processed as described by Shibata et al. (1989b) for assessment of BrdU incorporation, but in the urinary bladder rather than in the kidney. During week 4, urine samples were taken for urinalysis. At terminal sacrifice, urinary bladder tissues were processed for scanning electron microscopic examinations. There were no treatment-related deaths or adverse clinical signs. Although food and water consumption were similar to controls, biphenyl-treated rats showed a consistent reduction in average body weight (229 versus 247 g after 4 weeks and 300 versus 327 g after 8 weeks, for treated versus controls, respectively [$p < 0.01$]). A greater than fourfold increase in the BrdU labeling index was observed in urinary bladder epithelium of the biphenyl-fed rats (mean percent labeling index of 0.58 ± 0.31 compared to 0.13 ± 0.09 in controls; $p < 0.05$). Urinalysis revealed numerous microcalculi in the urinary sediment of the biphenyl-treated rats. This condition, designated as "severe" by the authors, was associated with histopathological lesions of the epithelium of the urinary bladder that included simple hyperplasia with moderate severity (5/5 rats), moderate pleomorphic microvilli (5/5 rats), moderate uniform microvilli (5/5 rats), and the occurrence of ropey or leafy microridges (5/5 rats), the latter condition designated as severe. Scanning electron microscope images of the luminal surface of bladder epithelial cells showed pleomorphic microvilli that varied in size and shape and the formation of microridges.

4.4.3. Biphenyl as a Tumor Promoter

Male B6C3F$_1$ mice (10–20/group) received the bladder carcinogen N-butyl-N (4-hydroxybutyl)nitrosamine (BBN) at 0 or 0.05% in the drinking water for 4 weeks followed by 0 or 1% biphenyl in the feed for 32 weeks (Tamano et al., 1993). The mice were observed for clinical signs, and body weight and food consumption were monitored. At 37-week terminal sacrifice, kidneys and urinary bladders were prepared for histopathological examination. No treatment-related clinical signs were observed. Mean body weight of the BBN + 1% biphenyl-treated mice was significantly ($p < 0.01$) lower than that of mice receiving BBN treatment only (32.2 ± 1.8 versus 38.4 ± 2.6 g). Biphenyl treatment did not result in increased incidences of simple hyperplasia or papillary or nodular dysplasia in the BBN-initiated mice. Administration of 1% biphenyl in the feed to eight mice for 8 weeks did not significantly affect indices of cell proliferation (BrdU incorporation) in urinary bladder epithelium.

In the initiation-promotion portion of a chronic toxicity study designed to assess the ability of biphenyl to promote carcinogenesis by EHEN in the kidney (see Section 4.2.1.2.1 for a detailed study description), male Wistar rats (25/group) received a basal diet with either 0 or 0.1% dietary EHEN for 2 weeks, followed by a basal diet containing either 0, 0.125, or 0.5% biphenyl for 34 weeks (Shiraiwa et al., 1989). At terminal sacrifice, gross pathologic

examinations were performed. Kidney and urinary bladder were fixed; kidneys were sectioned transversely (10–12 serial slices) and urinary bladders were cut into 4–6 serial slices. The authors used a computer-linked image analyzer to determine the incidence of kidney lesions and dysplastic foci. The presence of stones in the kidney and urinary bladder was assessed qualitatively using an infrared spectrophotometer.

Based on reported values for mean daily biphenyl intake (mg biphenyl/rat) and average body weight (mean initial body weight + one-half the difference between mean initial and mean final body weight) for each study group, doses of biphenyl at the 0.125 and 0.5% dietary levels are estimated to have been 59.28 and 248.3 mg/kg-day, respectively, for rats on basal diet alone for the first 2 weeks and 62.0 and 248.2 mg/kg-day, respectively, for rats on basal diet and EHEN for the first 2 weeks. Stones were present in the kidney, ureter, and urinary bladder of high-dose rats irrespective of whether animals were initially exposed to the basal or EHEN-containing diet (combined incidences of 6/25 and 8/25, respectively). The incidence of rats with renal cell tumors after EHEN and subsequent biphenyl administration was lower than that of rats receiving EHEN followed by basal diet (7/25 and 13/25, respectively). This finding indicates that biphenyl was not a promoter of renal cell tumors in male Wistar rats under the conditions of the study.

Male F344 rats (25/group) were exposed to 0.05% BBN (a bladder carcinogen) in the drinking water for 4 weeks followed by diets containing either 0 or 0.5% biphenyl for 32 weeks (Kurata et al., 1986). One group of five rats received biphenyl without pretreatment with BBN. The rats receiving biphenyl either with or without pretreatment with BBN gained less weight than control rats or those receiving only BBN. Incidences of urinary bladder hyperplasia, papilloma, and carcinoma were 17/18 (94%), 15/18 (83%), and 11/18 (61%), respectively, in the group of rats that survived treatment of BBN followed by biphenyl, compared to 6/24 (25%), 3/24 (12%), and 0/24 (0%), respectively, in the rats receiving BBN only. These urinary bladder lesions were not seen in any of the five rats receiving biphenyl without BBN pretreatment. Urinary bladder calculi were found in 25% of the rats receiving BBN followed by biphenyl and in 12% of the rats receiving BBN only. Biphenyl was considered a urinary bladder tumor promoter in male F344 rats under the conditions of the study.

Biphenyl was negative for tumor promotion in a skin-painting experiment in which the initiator was 0.3% 9,10-dimethyl-1,2-benzanthracene in benzene (Boutwell and Bosch, 1959). In the 16/20 mice that survived the topical application of 20% biphenyl for 16 weeks, none had developed papillomas or carcinomas as a result of treatment.

Six-week-old male F344 rats (20–30/group) were exposed to BBN in drinking water at 0.01 or 0.05% for 4 weeks, followed by 0.5% biphenyl in the feed for 32 weeks (Ito et al., 1984). Controls receiving only BBN and controls receiving only biphenyl were included. After sacrifice, urinary bladders were prepared for light microscopic assessment of neoplastic and

cancerous lesions. The study authors reported that biphenyl exhibited moderate bladder cancer-promoting activity, but data to support this finding were not included in the study report.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

Studies have been conducted to investigate the mechanisms by which biphenyl induces effects on the urinary tract, liver, and endocrine system. Other studies have looked at the potential for biphenyl to induce apoptosis, to affect mitochondrial activity, and to induce genetic changes. This literature is summarized in Appendix B. Mechanistic studies of biphenyl effects on the urinary tract, a principal target of biphenyl toxicity, and genotoxic potential are briefly discussed below.

4.5.1. Effects on the Urinary Tract of Rats

Mechanistic studies in F344/N rats have been performed to identify urinary metabolites of biphenyl, to assess conditions leading to calculi formation, and to determine the composition of urinary crystals and calculi (Ohnishi et al., 2001; 2000a; 2000b). Urinary calculi in male F344/N rats exposed to 4,500 ppm biphenyl in the diet for 104 weeks have been shown to be composed mainly of 4-hydroxybiphenyl-O-sulphate, whereas calculi in female rats were composed primarily of 4-hydroxybiphenyl and potassium sulphate, the hydrolysis products of 4-hydroxybiphenyl-O-sulphate (Ohnishi et al., 2000b). Using a study design that also involved the addition of potassium bicarbonate, potassium chloride, or sodium bicarbonate to the diet for 13 weeks, Ohnishi et al. (2001) determined that a combination of high urinary pH and high potassium levels was necessary to cause precipitation of biphenyl sulphate, and proposed that the crystalline precipitate caused obstruction that led to damaged of the transitional epithelium in the urinary bladder.

4.5.2. Genotoxicity

The overall weight of evidence for biphenyl genotoxicity from short-term tests is negative or equivocal (see Appendix B, Table B-2). Biphenyl did not induce mutations in a variety of bacterial test systems (in the absence or presence of exogenous metabolic activation), but in vitro assays of genotoxicity in mammalian test systems yielded a mix of negative and positive results, with positive results mostly in the presence of metabolic activation. In tests of clastogenic effects in mammalian systems, biphenyl induced SCEs, CAs, and micronuclei in cultured human peripheral blood lymphocytes (Rencüzoğullari et al., 2008) and CAs in one assay of CHL fibroblasts in the presence, but not the absence, of rat liver metabolic activation (Sofuni et al., 1985). However, biphenyl did not induce clastogenic effects (in the presence of metabolic activation) in other assays with Chinese hamster fibroblasts (Ishidate et al., 1984;

Ishidate and Odashima, 1977) or CHO cells (Yoshida et al., 1978). In the only adequately reported in vivo genotoxicity studies with biphenyl, single oral doses of 2,000 mg/kg of biphenyl or 2-hydroxybiphenyl induced DNA damage in several organs of CD-1 mice (including liver and bladder), but it is uncertain if the damage was due to a direct effect on DNA by biphenyl or its metabolites or indirectly due to cytotoxicity or ROS generated by redox cycling of a hydroquinone metabolite of 2-hydroxybiphenyl (Sasaki et al., 2002; Sasaki et al., 1997).

The overall weight of evidence for 2-hydroxybiphenyl genotoxicity (see Appendix B, Table B-3) suggests that oxidative DNA damage from redox cycling between 2,5-dihydroxybiphenyl and phenylbenzoquinone is possible (Sasaki et al., 2002; Sasaki et al., 1997; Pathak and Roy, 1993; Morimoto et al., 1989), but no evidence for DNA adducts or DNA binding in urinary bladder epithelium tissue was found in rats following short-term (Kwok et al., 1999) or subchronic (Smith et al., 1998) oral exposure to 2-hydroxybiphenyl at high doses associated with the formation of urinary bladder tumors. Increased micronuclei in urinary bladder epithelium were detected in rats exposed to 2% 2-hydroxybiphenyl or its sodium salt in the diet for 14 days (Balakrishnan et al., 2002). The mechanism of this clastogenic effect is uncertain, but could involve micronuclei formation in secondary response to cytotoxicity or regenerative cell proliferation, DNA damage from ROS generated from redox cycling of a hydroquinone metabolite, or protein modifications leading to mitotic spindle interference or inhibition of enzymes important in DNA replication.

4-Hydroxybiphenyl, the predominant metabolite of biphenyl, was not mutagenic in bacterial testing at noncytotoxic concentrations (Narbonne et al., 1987; Hanada, 1977) (see Appendix B, Table B-3). 2,5-Dihydroxybiphenyl (i.e., phenylhydroquinone) caused in vitro damage to human DNA from plasmid pbcNI in the presence of Cu(II) (Inoue et al., 1990) and DNA adducts when applied to mouse skin (Pathak and Roy, 1993), but did not cause DNA damage when injected intravesically into the urinary bladder of F344 rats at a concentration of 0.05% (Morimoto et al., 1989).

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

Tables 4-14 and 4-15 include the major studies and the observed effects for oral and inhalation exposure to biphenyl, respectively.

Table 4-14. Summary of major studies evaluating effects of biphenyl after oral administration in rats and mice

Species, strain	Exposure route	Dose (mg/kg-d), duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effect(s) at the LOAEL	Comments	Reference
Subchronic studies							
Rat, Long-Evans (female, 8/group)	Diet	0, 10, 30, or 100 90 d	ND	ND	Lower average plasma BUN levels in all exposed groups (statistical significance not reported and biological significance is uncertain).		Dow Chemical Co. (1953)[a]
Mouse, BDF_1 (10/sex/group)	Diet	0, 93, 347, 747, 1,495, 1,868, or 2,989 13 wks	M: 747 F: 747	M: 1,495 F: 1,495	M: Decreased body weight. F: Decreased body weight	To overcome possible problems with taste aversion, animals in the 3 highest dose groups received lower doses for the first 1-2 wks of exposure followed by the final dose for the remaining time.	Umeda et al. (2004b)
Chronic studies							
Rat, F344 (50/sex/group)	Diet	M: 0, 36.4, 110, or 378 F: 0, 42.7, 128, or 438 2 yrs	M: 110 F: 42.7	M: 378 F: 128	M: Bladder tumors and transitional cell hyperplasia. F: Nonneoplastic kidney lesions (transitional cell hyperplasia in the renal pelvis and hemosiderin deposits).		Umeda et al. (2002)

Table 4-14. Summary of major studies evaluating effects of biphenyl after oral administration in rats and mice

Species, strain	Exposure route	Dose (mg/kg-d), duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effect(s) at the LOAEL	Comments	Reference
Rat, Wistar (50/sex/group)	Diet	M: 0, 165, or 353 F: 0, 178, or 370 75 wks	M: ND F: ND	M: 165 F: 178	Formation of kidney stones associated with pyelonephritis in both sexes.		Shiraiwa et al. (1989)
Rat, Wistar (male, 25/group)	Diet	Control groups: basal diet for 2 wks followed by exposure at 0, 59.28, or 248.3 for 34 wks Exposure groups: diet containing 0.1% EHEN for 2 wks followed by 0, 62, or 248.2 for 34 wks	Control: 59.28 Exposure: 62	Control: 248.3 Exposure: 248.2	Formation of kidney stones associated with pyelonephritis in both groups.	Biphenyl did not exhibit tumor promoting characteristics for the kidney tumor initiator, EHEN, under the conditions of this study.	Shiraiwa et al. (1989)
Rat, albino (weanling, 15/sex/group)	Diet	0, 1, 4, 8, 42, 84, 420, and 840 2 yrs	84	420	Kidney effects including tubular atrophy and dilation associated with cyst formation and calculi formation in the renal pelvis of both sexes.		Ambrose et al. (1960)
Rat, albino (male, 8/group)	Diet	0, 250, or 450 13 mo	ND	250	Nonneoplastic degenerative changes in the liver, kidney, thyroid, and parathyroid resulting in hyperplasia of liver, kidney, and thyroid.		Pecchiai and Saffiotti (1957)
Rat, Sprague-Dawley (12/sex/group)	Diet	0, 7, 73, or 732 2 yrs	73	732	Renal effects (tubular dilatation, calcification, and intratubular inflammation).	Decreased survival and small number of animals/group may have impaired ability to detect late-developing tumors.	Dow Chemical Co. (1953)[a]

Table 4-14. Summary of major studies evaluating effects of biphenyl after oral administration in rats and mice

Species, strain	Exposure route	Dose (mg/kg-d), duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effect(s) at the LOAEL	Comments	Reference
Mouse, BDF_1 (50/sex/group)	Diet	M: 0, 97, 291, or 1,050 F: 0, 134, 414, or 1,420 2 yrs	M: 97 F: 134	M: 291 F: 414	M: Decreased body weight. F: Nonneoplastic effects (mineralization in the kidney and significantly increased plasma ALT and AST activities) in female mice.		Umeda et al. (2005)
Mouse, ddY (female, 34–37/group)	Diet	0 or 855 2 yrs	855	ND	No adverse effects observed at the dose tested.		Imai et al. (1983)
Mice, hybrid (two strains, 18/sex/strain/group)	Gavage (215 mg/kg body weight in 0.5% gelatin) for the first 3 wks, followed by dietary exposure for the remaining time	0 or 91 18 mo	91	ND	No evidence of a carcinogenic response.	Two strains of F1 hybrid mice were produced by mating female C57BL/6 mice with either male C3H/Anf mice or male AKR mice.	Innes et al. (1969); NCI (1968)
Dog, Mongrel (males/group; 1 female/group)	Capsule in corn oil	0, 2.5, or 25 5 d/wk for 1 yr	ND	ND	ND		Monsanto (1946)[a]
Monkey, Rhesus (2 males/group; 1female/group)	Diet	0, 0.01, 0.1, or 1% for 1 yr	ND	ND	ND	Author considered an increase in relative liver weight in high-dose monkeys to be possibly compound-related	Dow Chemical Co. (1953)[a]

Table 4-14. Summary of major studies evaluating effects of biphenyl after oral administration in rats and mice

Species, strain	Exposure route	Dose (mg/kg-d), duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effect(s) at the LOAEL	Comments	Reference
Reproductive and developmental studies							
Rat, Wistar (18–20 pregnant females/group)	Gavage in corn oil	0, 125, 250, 500, or 1,000 on GDs 6–15	Dam: 500 Offspring: 250	Dam: 1,000 Offspring: 500	Dam: Maternal toxicity (increased mortality), increased in dead or resorbed fetuses. Offspring: Increased incidence of anomalous fetuses and litters.		Khera et al. (1979)
Rat, Long Evans (3 males/group; 9 females/group)	Diet	M: 9, 89, or 887 F: 10, 101, or 1,006 Continuous breeding	M: ND F: 101	M: ND F: 1,006	M: ND F: Decreased fertility and litter size; reduced offspring body weight.	The effects seen in the high-dose group may be associated with unpalatability and resultant decreased food intake.	Dow Chemical Co.(1953)[a]
Rat, albino Experiment 1: 3-5 males/group; 9-10 females/group. Experiment 2: 3-4 males/group; 8-9 females/group.	Diet	0, 105, or 525 Experiment 1: 60 days prior to mating Experiment 2: 11 days prior to mating	ND	ND	ND	Authors presented tabulated data and concluded that the compound had no significant effect on reproduction.	Ambrose et al. (1960)

[a]Report was not peer reviewed.

F = female; M = male; ND = not determined

Note: Other studies of subchronic duration that examined the effects of biphenyl on the urinary tract only (Shibata et al., 1989a; Shibata et al., 1989b) are summarized in Section 4.4.2. Because these studies were designed to investigate the effects of biphenyl on the kidney and urinary bladder and the mode of action by which biphenyl induces these effects, the studies were not useful for identifying NOAELs and LOAELs, and were not included in this table.

Table 4-15. Summary of major studies evaluating effects of biphenyl after inhalation exposure in rats, mice and rabbits

Species, strain	Dose (mg/m^3), duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Effect(s) at the LOAEL	References
Rabbit, albino (3/group) Rat, Sprague-Dawley (10/group)	300 mg/m^3 (7 hrs/d, 5 d/wk) 64 d over 94-d period	Rabbit: ND Rat: ND	Rabbit: ND Rat: 300	Rabbit: ND Rat: Mortality (5/10), acute emphysema, congestion, edema, bronchitis, lobular pneumonia, and multiple pulmonary abscesses	Deichmann et al. (1947); Monsanto (1946)
Rabbit, albino (3/group) Rat, Sprague-Dawley (6/group)	40 mg/m^3 (7 hrs/d, 5 d/wk) 46 d over 68-d period	Rabbit: ND Rat: ND	Rabbit: ND Rat: 40	Rabbit: ND Rat: Mortality (1/6), acute emphysema, congestion, edema, bronchitis, lobular pneumonia, and multiple pulmonary abscesses	
Mice (12/group) Rat, Sprague-Dawley (4/group)	5 mg/m^3 (7 hrs/d, 5 d/wk) 62 d over 92-d period	Mouse: ND Rat: ND	Mouse: 5 Rat: ND	Mouse: Mortality (2/12); upper respiratory tract irritation (acute emphysema, congestion, edema, bronchitis, lobular pneumonia, and multiple pulmonary abscesses) Rat: ND	
Mouse, CDI (50/sex/group)	0, 157.7, or 315.3 mg/m^3 (7 hrs/d, 5 d/wk), 13 wks	ND	157.7	Histopathologic lung, liver, and kidney lesions (congested and hemorrhagic lungs, tracheal hyperplasia, and congestion and edema in the liver and kidney) in both sexes.	Sun Company Inc. (1977b)[a]

[a]Report was not published.

ND = not determined

4.6.1. Oral

Biphenyl displays a relatively low acute oral toxicity, with LD_{50} values in laboratory animals in the 2–3 g/kg range (see Section 4.4.1). The major symptoms of biphenyl intoxication typically associated with short-term, high-dose oral exposure of animals are labored breathing, loss of body weight, and weakness. Following medium- or long-term oral exposure, consistent findings indicated reduced body weight gain (Umeda et al., 2005; Umeda et al., 2004b; Umeda et al., 2002; Ambrose et al., 1960; Dow Chemical Co, 1953) and increased liver and kidney weights (Umeda et al., 2004b; Umeda et al., 2002; Søndergaard and Blom, 1979; Ambrose et al., 1960; Dow Chemical Co, 1953; Monsanto, 1946) in rodents. In some studies, reduced weight gain has been attributed to low palatability of the feed by the authors (Ambrose et al., 1960; Dow Chemical Co, 1953); however, the feed intake data of Umeda et al. (2005) in mice did not support this hypothesis. Signs of liver damage (increased serum activities of ALT, AST, AP, and LDH) were observed in mice (Umeda et al., 2005).

Pathological effects on the urinary system have been reported in dogs (Monsanto, 1946), rats (Umeda et al., 2002; Dow Chemical Co, 1953), and mice (Umeda et al., 2005). Increased urine volume with increased specific gravity, polycystic changes, nephritis, and precipitation of free 4-OH-biphenyl and its glucuronide in urine have been consistently reported following oral exposure to biphenyl (Kluwe, 1982; Søndergaard and Blom, 1979; Booth et al., 1961; Monsanto, 1946). Calculi appeared in the urine of male rats only (Umeda et al., 2002; Ohnishi et al., 2001; Ohnishi et al., 2000a; Ohnishi et al., 2000b; Shibata et al., 1989b; Ambrose et al., 1960). Urothelial hyperplasia with increased indices of cell proliferation have been described in rats but not in mice and were attributed to irritation by calculi (Umeda et al., 2005; Umeda et al., 2002; Shibata et al., 1989b). Tubular dilatation and morphological changes in papillae and pelvis, kidney stones, obstructive pyelonephritis, tubular atrophy, fibrosis, and pelvic hyperplasia were observed (Shibata et al., 1989b; Shibata et al., 1989a; Shiraiwa et al., 1989; Takita, 1983; Kluwe, 1982; Booth et al., 1961).

Increased incidences of fetuses with skeletal anomalies were reported following gavage administration of biphenyl to Wistar rats during gestation (Khera et al., 1979). A three-generation study in rats (Dow Chemical Co, 1953) found general reproductive toxicity at high doses (about 947 mg/kg-day).

4.6.2. Inhalation

In a case study of workers engaged in the production of biphenyl-impregnated paper, Häkkinen et al. (1973; 1971) observed liver damage (elevated levels of serum AST and ALT; incipient cirrhosis and fatty changes in biopsy specimens) and effects on the central and peripheral nervous systems (polyneuritic symptoms [abnormal EEGs and ENMGs], giddiness, fatigue) that were attributed to long-term exposure to high concentrations of biphenyl. In one fatal case, autopsy revealed kidney and bone marrow damage and heart muscle degeneration, as

well as brain edema (Häkkinen et al., 1973; Häkkinen et al., 1971). A small cluster of Parkinson's disease (PD) was reported at a facility manufacturing biphenyl-impregnated paper, but other studies have not found a similar association (Wastensson et al., 2006). The workplace conditions reported for these studies (Wastensson et al., 2006; Häkkinen et al., 1973; Häkkinen et al., 1971) suggested that inhalation represented the predominant route of exposure, but dermal absorption as well as oral uptake (hand to mouth) might have occurred at a significant level.

In mice, short-term biphenyl inhalation at concentrations as high as 54.75 ppm (345.5 mg/m^3) appeared to cause no observable clinical toxicity (Sun, 1977b). In another study, groups of rabbits, rats, or mice were exposed to biphenyl by inhalation for 7–13 weeks at concentrations ranging from 5 to 300 mg/m^3 (Deichmann et al., 1947). No adverse effects were observed in rabbits, while rats and mice showed irritation of mucous membranes and succumbed at high concentrations. Mice were more sensitive than rats in these experiments, additionally showing congestion and hemorrhage of the lungs (Deichmann et al., 1947). High incidences of pneumonia and tracheal hyperplasia, and congestion and edema in the lungs, liver, and kidney were reported in a 13-week inhalation study of biphenyl in mice that was limited by study methodologic and reporting issues (Sun, 1977a). Reproductive or developmental studies using the inhalation route of exposure were not identified.

4.6.3. Mode-of-Action Information

The studies described above have demonstrated that exposure to biphenyl may lead to a variety of noncancer health effects (i.e., weight loss, liver toxicity, urinary tract toxicity). However, the available information is insufficient to establish the mode of action for noncancer health effects following exposure to biphenyl.

Weight loss or lack of weight gain has been consistently associated with oral exposure to biphenyl (Umeda et al., 2005; Umeda et al., 2002; Shibata et al., 1989b; Ambrose et al., 1960; Dow Chemical Co, 1953). The work of Nishihara (1985) provides a possible explanation for this toxic effect. This author found that, in vitro, biphenyl can act as an uncoupler of respiration. It may be speculated that long-term, high-dose exposure to biphenyl uncouples mitochondrial respiration to a certain extent, resulting in a futile cycle that diverts the use of nutrients from building body mass into maintaining necessary energy stores. It is not clear at what level of in vivo exposure this effect might become operative.

Several of the oral animal studies (Umeda et al., 2005; Sun, 1977a; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953; Deichmann et al., 1947) and the epidemiological study by Häkkinen et al. (1973) provide evidence that the liver is a target for biphenyl toxicity. This evidence consists of changes in blood parameters that are indicative of liver toxicity; however, in animal studies, liver histopathology does not support or explain this finding. Evidence for damage to the nervous system was suggested in epidemiology studies by Häkkinen et al. (1973) and Seppäläinen and Häkkinen (1975). The nervous system has not been identified as a target in

chronic toxicity studies in rodents. The limited evidence for an estrogenic activity of 4,4′-dihydroxybiphenyl (Kitamura et al., 2003; Schultz et al., 2002) is insufficient to assign a clear endocrine-disrupting effect to this major metabolite of biphenyl.

Damage to the urinary tract has been observed consistently in animals. The work of Ohnishi et al. (2001; 2000a; 2000b) provides evidence that, in the rat, this is due to the precipitation in the urinary tract of crystals consisting mostly of 4-hydroxybiphenyl. These crystals irritate the epithelia of ureters and bladder, leading to chronic inflammation and obstruction of the urinary tract with subsequent hydronephrosis. The work of Ohnishi et al. (2001; 2000b) has made it clear that, at least in their animal model, two conditions are required for this event to occur: (1) the pH in the urine of the animals needs to be elevated and (2) elevated potassium levels need to accompany the elevated pH because it is the potassium salt of 4-hydroxybiphenyl sulphate that has the lowest solubility in high-pH urine.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for biphenyl provides "suggestive evidence of carcinogenic potential" at environmentally relevant exposure levels in humans where the formation of urinary bladder tumors would not be expected to occur. This cancer descriptor is based on an increase in the incidence of liver tumors (hepatocellular adenomas and carcinomas) in female BDF_1 mice (Umeda et al., 2005) and urinary bladder tumors (transitional cell papillomas and carcinomas) in male F344 rats (Umeda et al., 2002) exposed to biphenyl in the diet for 104 weeks, as well as information on mode of carcinogenic action. Earlier chronic cancer bioassays in orally exposed animals found no clear evidence of biphenyl-induced carcinogenicity in rats (Shiraiwa et al., 1989; Ambrose et al., 1960; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953), mice (Imai et al., 1983; Innes et al., 1969; NCI, 1968), dogs (Monsanto, 1946), or Rhesus monkeys (Dow Chemical Co, 1953). The findings from these earlier studies were less informative for the carcinogenicity of biphenyl than Umeda et al. (2005, 2002) because of various study limitations. With the exception of Imai et al. (1983), these limitations include small group sizes and shorter-than-lifetime exposure durations due to design or decreased survival unrelated to tumor development. Imai et al. (1983) found no evidence of carcinogenic responses in female mice of a different species (ddY mice) (n = 34) exposed to 5,000 ppm biphenyl in the diet for 2 years (Imai et al., 1983).

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) ("Cancer Guidelines") emphasize the importance of weighing all of the evidence in reaching conclusions about the human carcinogenic potential of agents. Information on mode of action has been taken into consideration in evaluating the weight of evidence for carcinogenicity. The induction of urinary bladder tumors in F344 male rats by dietary biphenyl exposure is a high-dose

phenomenon closely related to the formation of urinary bladder calculi. As discussed in more detail in Section 4.7.3.1, the mode of action information is sufficient to conclude that urinary bladder tumors will not occur without the development of calculi. While the proposed mode of action for urinary bladder tumors in male rats is assumed to be relevant to humans, the available evidence suggests that humans would be less susceptible to these tumors than rats (see discussion in Section 4.7.3.1.4.2). Overall, the mode of action analysis supports the conclusion that biphenyl should not pose a risk of urinary bladder tumors at environmentally relevant exposure levels in humans.

The available data are insufficient to establish a mode of action for liver tumors in female mice (see Section 4.7.3.2.2.1 for further discussion). In the absence of information to indicate otherwise, the development of liver tumors in female mice with chronic exposure to biphenyl is assumed to be relevant to humans. EPA acknowledges that some mouse strains are relatively susceptible to liver tumors and the background incidence of this tumor can be high. For these reasons, use of mouse liver tumor data in risk assessment has been a subject of controversy (King-Herbert and Thayer, 2006). According to historical control data from the Japan Bioassay Research Center, the institute that conducted the mouse bioassay published by Umeda et al. (2005), the mean incidence of liver tumors (hepatocellular adenoma or carcinoma) in male and female control BDF_1 mice is 32.2 and 7.1%, respectively, incidences consistent with the concurrent controls in the mouse bioassay of biphenyl. The relatively low background incidence of liver tumors in female control mice from Umeda et al. (2005) minimizes the possible confounding of compound-related liver tumors in this sex.

Thus, when one takes into consideration information on the mode of action for biphenyl-induced tumors, risk of female liver tumors only is operative at environmentally relevant exposures. Accordingly, this assessment concludes that there is "suggestive evidence of carcinogenic potential."

EPA's Cancer Guidelines (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the cancer descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of biphenyl demonstrates that tumors occur in tissues remote from the site of absorption following chronic oral exposure (urinary bladder in male rats and liver in female mice). No information on the carcinogenic effects of biphenyl via the inhalation or dermal routes in humans and animals is available. Biphenyl is rapidly and extensively absorbed by the oral route of exposure, but no studies of uptake following inhalation or dermal exposure have been conducted; however, a case report of hepatic toxicity produced by a probable combination of inhalation and dermal exposures in a worker in a biphenyl-impregnated fruit wrapping paper production facility (Häkkinen et al., 1973) provides qualitative evidence of absorption by these routes. Therefore, based on the observation of systemic tumors

following oral exposure and assumed absorption by all routes of exposure, it is assumed that an internal dose will be achieved regardless of the route of exposure. In the absence of information to indicate otherwise, the database for biphenyl provides "suggestive evidence of carcinogenic potential" by all routes of exposure.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

Available human studies were not designed to evaluate associations between exposure to biphenyl and occurrence of cancer (see Section 4.1).

As discussed in Section 4.2, carcinogenicity studies in animals are limited to the oral exposure route. In well-designed cancer bioassays of F344 rats (Umeda et al., 2002) and BDF_1 mice (Umeda et al., 2005), dietary exposure to biphenyl resulted in the occurrence of urinary bladder tumors in male rats and significantly increased incidences in liver tumors in female mice.

Earlier chronic toxicity and carcinogenicity assessments found no clear evidence of biphenyl-induced carcinogenicity in orally exposed rats, mice, dogs, or Rhesus monkeys. However, these studies were generally limited in design, with the exception of a study reporting no evidence of carcinogenic responses in female ddY mice (n = 34 mice versus 37 control mice) exposed to 5,000 ppm biphenyl in the diet for 2 years (Imai et al., 1983). In a study of Wistar rats, sufficient numbers of animals (50/sex/group) were exposed to biphenyl in the diet at concentrations up to 5,000 ppm, but only for 75 weeks (Shiraiwa et al., 1989). Some of the male rats exhibited urinary bladder calculi and simple or diffuse hyperplasia and papillomatosis of the urinary bladder mucosa in the absence of neoplastic lesions, but the study may have been terminated prior to eventual urinary bladder tumor development. Ambrose et al. (1960) exposed albino rats (15/sex/exposure level) to biphenyl in the diet at concentrations up to 10,000 ppm for 2 years (10, 50, 100, 500, 1,000, 5,000, or 10,000 ppm); however, decreased survival in rats exposed to 5,000 or 10,000 ppm, presumably from decreased food consumption, and the relatively small numbers of animals in each exposure group may have impaired the ability to detect late-developing tumors. In another study, groups of Sprague-Dawley rats (12/sex/group) received biphenyl in the diet at concentrations up to 10,000 ppm for up to 2 years (Dow Chemical Co, 1953). However, this study suffered from a pneumonia outbreak, particularly among control males, and the relatively small numbers of animals and the decreased survival may have impaired the ability to detect late-developing tumors. A study of male albino rats included small numbers of rats (8/group) and a short (13 months) exposure period (Pecchiai and Saffiotti, 1957). A study of $B6C3F_1$ or $B6AkF_1$ mice exposed to biphenyl in the diet for only 18 months (Innes et al., 1969; NCI, 1968) included relatively small numbers of mice (18/sex/group) and only one exposure level (517 ppm) that was similar to the concentration (667 ppm) without carcinogenic effect in the Umeda et al. (2005) 24-month BDF_1 mouse bioassay. The dog study included two males and one female, a high dose of 25 mg/kg-day, and an exposure period of only 1 year (Monsanto, 1946). Rhesus monkeys (two males and one

female) were exposed to biphenyl in the diet at a concentration of 10,000 ppm, but for only 1 year (Dow Chemical Co, 1953).

The overall weight of evidence for biphenyl genotoxicity from short-term tests is negative or equivocal. Biphenyl did not induce mutations in a variety of bacterial test systems, but both negative and positive results were obtained in mammalian in vitro test systems (see Section 4.5.6). Single oral doses of 2,000 mg biphenyl/kg induced DNA damage (detected by the Comet assay) in several organs of CD-1 mice (including the liver and bladder), but it is uncertain if the damage was due to a direct effect on DNA or was an indirect effect due to cytotoxicity or ROS generated by redox cycling of phenylhydroquinone, a major urinary metabolite of 2-hydroxybiphenyl and a minor metabolite of biphenyl in rats (Sasaki et al., 2002; Smith et al., 1998).

The overall weight of evidence for 2-hydroxybiphenyl genotoxicity suggests that oxidative DNA damage from ROS from redox cycling between 2,5-dihydroxybiphenyl and phenylbenzoquinone is possible. DNA damage was detected in liver and bladder of CD-1 mice exposed to 2,000 mg/kg of 2-hydroxybiphenyl (Sasaki et al., 2002, 1997) and in the urinary bladder of male F344 rats fed the sodium salt of 2-hydroxybiphenyl at 1 or 2% in the diet for 3–5 months (Morimoto et al., 1989). DNA adducts were detected by [^{32}P]-post labeling in skin of CD-1 mice after topical application of the sodium salt of 2-hydroxybiphenyl or phenylhydroquinone (Pathak and Roy, 1993), and increased micronuclei were detected in urinary bladder epithelium of male F344 rats exposed to 2,000 ppm 2-hydroxybiphenyl or 2,000 ppm NaCl plus 2,000 ppm 2-hydroxybiphenyl in the diet for 2 weeks (Balakrishnan et al., 2002). However, increased binding of radioactivity to DNA was not detected in DNA extracted from urinary bladder epithelium of male F344 rats exposed to single gavage doses of 2-hydroxybiphenyl as high as 1,000 mg/kg (Kwok et al., 1999), and DNA adducts were not detected in urinary bladder epithelium of male F344 rats exposed for 13 weeks to biphenyl dietary concentrations as high as 12,500 ppm (Smith et al., 1998). The mechanism by which 2-hydroxybiphenyl may induce micronuclei in the urinary bladder epithelium is uncertain, but could involve micronuclei generation as a secondary response to cytotoxicity or regenerative cell proliferation, DNA damage from ROS from redox cycling of 2,5-dihydroxybiphenyl, or protein modifications leading to mitotic spindle interference or inhibition of enzymes important in DNA replication (Balakrishnan et al., 2002). The hydroxylation of biphenyl to produce 2-hydroxybiphenyl is a minor pathway in rats and mice (Halpaap-Wood et al., 1981a, b; Meyer and Scheline, 1976). 2-Hydroxybiphenyl and 2,5-dihydroxybiphenyl collectively accounted for <2% of metabolites in urine of rats administered single oral doses of 100 mg biphenyl/kg (Meyer and Scheline, 1976) or single i.p. doses of 30 mg biphenyl/kg (Halpaap-Wood et al., 1981b). In mice given i.p. doses of 30 mg biphenyl/kg, these metabolites accounted for <5% of urinary metabolites (Halpaap-Wood et al., 1981b).

4.7.3. Mode-of-Action Information

4.7.3.1. *Mode-of-Action Information for Bladder Tumors in Male Rats*

4.7.3.1.1. *Hypothesized mode of action.* The best-supported hypothesis proposes a mode of action whereby the formation of urinary bladder calculi (from the precipitation of 4-hydroxybiphenyl-O-sulphate) is a key event in the development of urinary bladder tumors in male rats fed high levels of biphenyl in the diet for 2 years. According to this hypothesis, the calculi (occurring in association with increased urinary pH and potassium, and predominantly composed of 4-hydroxybiphenyl-O-sulphate) cause irritation to transitional epithelial cells of the urinary bladder leading to sustained cell proliferation, which promotes the development of initiated cells in the urinary bladder with progression to papillomas and carcinomas.

4.7.3.1.2. *Experimental support for the hypothesized mode of action*

Strength, consistency, and specificity of association, including support for the hypothesized mode of action in male rats. The formation of urinary bladder calculi, predominantly composed of potassium 4-hydroxybiphenyl-O-sulphate, is strongly, consistently, and specifically associated with the formation of urinary bladder tumors in male rats chronically exposed to high dietary concentrations of biphenyl. Several findings support this association. Urinary bladder calculi were formed at a high prevalence (43/50; 86%) in a group of male rats exposed to biphenyl in the diet at a concentration of 4,500 ppm, but were absent in male rats receiving diets containing 0, 500, or 1,500 ppm biphenyl (Umeda et al., 2002). These observations were consistent with the detection of urinary bladder transitional cell papilloma (10/50; 20%), carcinoma (24/50; 48%), and papilloma or carcinoma (31/50; 62%) in the 4,500 ppm group of male rats and total absence of urinary bladder papilloma or carcinoma in the control, 500, or 1,500 ppm groups of male rats. Bladder calculi were found in all 24 of the male rats with urinary bladder transitional cell carcinoma and in 8/10 of the male rats with transitional cell papilloma.

The association between urinary bladder calculus formation and development of urinary bladder tumors is both gender and species specific. Urinary bladder calculi, of similar size to those observed in males, were observed at much lower incidence (8/50; 16%) in the 4,500 ppm female rats, but they were of more uniform color (white and yellow versus white, yellow, brown, gray, and black in males) and shape (spheroidal versus triangular, pyramidal, cubical, and spheroidal in males) and primarily composed of 4-hydroxybiphenyl and potassium bisulphate (which are hydrolysis products of potassium 4-hydroxybiphenyl-O-sulphate) (Umeda et al., 2002; Ohnishi et al., 2000b). No urinary bladder calculi were found in the 500 and 1,500 ppm groups of female rats. Transitional cell hyperplasia was found in 10/50 4,500-ppm female rats, but no urinary bladder transitional cell papillomas or carcinomas were seen in any of the biphenyl-exposed groups of female rats. Furthermore, there was no evidence of biphenyl-induced urinary bladder calculi or bladder tumors in male or female BDF_1 mice receiving dietary biphenyl at concentrations as high as 6,000 ppm for 2 years (Umeda et al., 2005).

Urinary bladder calculi in male rats were associated with significantly increased urinary pH (average pH of 7.97 in the 4,500 ppm group at the final week of exposure compared to 7.66 in controls) (Umeda et al., 2002) and were composed primarily of potassium 4-hydroxybiphenyl-O-sulphate (Ohnishi et al., 2000b). The urine pH of female rats exposed to 4,500 ppm for 104 weeks (pH = 7.26) was not elevated compared with controls (pH = 7.29) (Umeda et al., 2002). From these observations, it appears that the formation of the calculi results from the precipitation of the potassium salt of the sulphate conjugate of 4-hydroxybiphenyl under the elevated pH conditions of the male rat urine. The mechanism responsible for increased urinary pH is unknown, although Ohnishi et al. (2001; 2000a; 2000b) proposed that gender differences in urinary conditions, such as pH and potassium concentrations, and sulphatase activities in kidneys, may be responsible for the gender differences in urinary calculi composition and formation and the subsequent development of urinary bladder tumors in male, but not female, F344 rats.

Relatively strong, consistent, and specific associations between calculi formation and transitional cell hyperplasia and between transitional cell hyperplasia and the development of transitional cell tumors in the urinary bladder have been shown in male F344 rats chronically exposed to high concentrations of biphenyl in the diet. Urinary bladder transitional cell hyperplasia (simple, nodular, papillary) occurred in 45/50 (90%) male rats receiving biphenyl in the diet for 2 years at the same dietary concentration (4,500 ppm) at which high prevalences of both urinary bladder calculi formation (43/50; 86%) and transitional cell tumors (31/50 62%) were observed (Umeda et al., 2002). Forty-two of the 45 male rats with urinary bladder transitional cell hyperplasia also exhibited urinary bladder calculi. In another study, evidence of biphenyl-induced calculi formation (microcalculi in the urine) and increased indices of urinary bladder transitional cell proliferation (greater than fourfold increase in BrdU incorporation) in male F344 rats has been reported following as little as 4–8 weeks of dietary exposure to 5,000 ppm biphenyl (Shibata et al., 1989b).

The most convincing evidence that degenerative changes in the urinary bladder epithelium lead to tumor formation is the site-concordance of associations between calculi formation in the urinary bladder, transitional cell proliferation, transitional cell hyperplasia, and transitional cell tumors (Umeda et al., 2002). In addition, the strong associations between urinary tract calculi formation, ulcerations or inflammation, and subsequent hyperplasia combined with repeated, high-level exposure to other chemicals that cause urinary bladder tumors in rodents, including melamine, uracil, and the sodium salt of 2-hydroxybiphenyl (Capen et al., 1999; IARC, 1999a; IARC, 1999b; Cohen, 1998, 1995) provide further evidence that degenerative changes are involved in the etiology of rodent urinary bladder tumors. It is not unusual to see extensive proliferation or hyperplasia in bladder epithelium in response to urinary calculi from other rodent bladder tumorigens without an associated ulceration or intense inflammatory response. In male rats exposed to 4,500 ppm biphenyl, increasing numbers of rats

with clinical hematuria were observed beginning at about the 40th week of exposure, and histologic examinations at study termination revealed focal hyperplasia in 45/50 rats, providing some evidence of calculi-induced bladder epithelial damage followed by cell proliferation (Umeda et al., 2002). Over the course of the study, 94% of male rats with hematuria had bladder or kidney calculi, but hematuria was not found in any biphenyl-exposed females. In addition, with 8 weeks, but not 4 weeks, of exposure to 5,000 ppm biphenyl in the diet, moderate urinary bladder epithelial hyperplasia and microcalculi in urine were observed in 5/5 male F344 rats, but no descriptions of degenerative changes were provided; these observations are consistent with a rapid repair response to epithelial damage from biphenyl-induced urinary tract calculi (Shibata et al., 1989b).

The ability of repeated biphenyl exposure to promote previously initiated urinary bladder cells to bladder tumors is supported by results of a bladder tumor initiation-promotion study (Kurata et al., 1986). Incidences of urinary bladder hyperplasia, papilloma, and carcinoma were significantly increased in male F344 rats initiated with dietary BBN for 4 weeks followed by 5,000 ppm biphenyl in the diet for 32 weeks, compared with rats receiving BBN only for 4 weeks. For example, 94 and 83% of rats treated with BBN followed by biphenyl developed urinary bladder hyperplasia and papillomas, respectively, compared with 25 and 12% of rats exposed to BBN alone.

The hypothesis that the mode of action involves the development of urinary bladder tumors in biphenyl-exposed male rats is further supported by an overall negative or equivocal weight of evidence for the genotoxicity of biphenyl. As discussed earlier, there are consistently negative results for biphenyl in bacterial mutation assays and inconsistent positive results for biphenyl in in vitro mammalian assays mostly in the presence of metabolic activation. There is evidence that 2,5-dihydroxybiphenyl (i.e., phenylhydroquinone), the principal urinary metabolite in rats exposed to high doses of 2-hydroxybiphenyl, can undergo redox cycling to produce ROS that may damage DNA and lead to tumor-initiating mutations; however, 2-hydroxybiphenyl is a minor urinary metabolite of biphenyl in rats and 2,5-dihydroxybiphenyl was not detected in urine of rats exposed to oral doses of 100 mg biphenyl/kg (Meyer and Scheline, 1976).

***Dose-response concordance*.** Dose-response relationships for urinary bladder calculi formation, transitional cell hyperplasia, and transitional cell tumor development show concordance in the 2-year oral study of rats (Umeda et al., 2002). In male rats, urinary calculi, nonneoplastic lesions (epithelial hyperplasia), and neoplastic lesions (papillomas and carcinomas) of the urinary bladder were observed only at the highest exposure level (4,500 ppm); no urinary bladder calculi, transitional cell hyperplasia, or transitional cell tumors were found in control, 500, or 1,500 ppm male rats. Furthermore, urinary bladder calculi were found in 43/45 high-dose male rats, in all 24 male rats with transitional cell carcinoma, and in 8/10 of the male rats with transitional cell papilloma.

Temporal relationship. Results from the 2-year oral study in rats (Umeda et al., 2002) provide some evidence of a progression from urinary bladder calculi formation to the development of bladder tumors. Urinary bladder calculi were observed in the first 4,500 ppm male rat that died (week 36), evidence of blood in the urine was observed in 4,500 ppm male rats by week 40, and incidences of bladder calculi and bloody urine that paralleled increases in mortality and tumor formation were observed throughout the remainder of the study. In addition, results of a short-term oral study demonstrate that microcalculi can be detected in the urine of male rats after as little as 4 weeks of dietary exposure to 5,000 ppm biphenyl and that hyperplasia of urinary bladder epithelium can be detected at least by week 8 (Shibata et al., 1989b). Presumably, the development of biphenyl-induced urinary bladder tumors requires a longer exposure period to urinary calculi of sufficient size, shape, and composition to induce urinary bladder epithelial damage and a sustained proliferative response.

Biological plausibility and coherence. The proposed mode of action is consistent with the current understanding of cancer biology and is supported by the wide body of evidence that other chemicals with primarily nongenotoxic profiles produce urinary bladder tumors in rodents at high exposure levels by a mode of action involving calculi formation, ulceration or inflammation, and regenerative cell proliferation (Capen et al., 1999; IARC, 1999a, b; Cohen, 1998, 1995). Additional information could strengthen the plausibility and coherence of the proposed mode of action to explain the occurrence of biphenyl-induced urinary bladder tumors in male rats. These additional data include results from investigations of earlier time points in the proposed temporal progression from calculi formation to epithelial damage, regenerative cell proliferation, and tumor development and further investigations into the factors underlying gender-specific differences in precipitation of 4-hydroxybiphenyl-O-sulphate to form bladder calculi in rats.

4.7.3.1.3. ***Other possible modes of action for bladder tumors in male rats.*** Although the weight of evidence from short-term standard genotoxicity tests with biphenyl and 4-hydroxybiphenyl is predominantly negative, evidence is available that suggests that oral exposure to high doses of 2-hydroxybiphenyl is associated with the development of urinary bladder tumors in male rats. The induction of genotoxic effects in the urinary bladder epithelium leading to tumor initiation is proposed to occur via redox cycling between 2,5-dihydroxy-biphenyl and phenylbenzoquinone (Balakrishnan et al., 2002; Kwok et al., 1999; Pathak and Roy, 1993; Morimoto et al., 1989). However, the strong, consistent, and specific association between the occurrence of urinary bladder calculi composed of 4-hydroxybiphenyl-O-sulphate and development of urinary bladder tumors in male but not female rats, the evidence that 2-hydroxybiphenyl is a minor urinary metabolite of biphenyl and, finally, that

2,5-dihydroxybiphenyl was not detected in the urine of biphenyl-exposed rats, demonstrate that the support for a genotoxic mode of action involving key mutational events from biphenyl or its metabolites in the urinary bladder leading to initiation of tumor cells is not compelling. Additional support for a proposed genotoxic mode of action would come from studies showing formation of 2,5-dihydroxybiphenyl and phenylbenzoquinone in the urinary bladder epithelium of rats exposed to low doses of biphenyl.

4.7.3.1.4. *Conclusions about the hypothesized mode of action for bladder tumors in male rats*

Support for the hypothesized mode of action in rats. There is strong evidence that urinary bladder tumors in male rats chronically exposed to biphenyl in the diet is a high-dose phenomenon involving sustained occurrence of calculi in the urinary bladder leading to transitional cell damage, sustained regenerative cell proliferation, and eventual promotion of spontaneously initiated tumor cells in the urinary bladder epithelium.

To summarize, chronic exposure of male rats to a high dietary concentration of biphenyl (4,500 ppm) caused increased urinary pH and high prevalence of urinary bladder calculi (from the precipitation of 4-hydroxybiphenyl-O-sulphate in the urine), transitional cell hyperplasia, and transitional cell tumors. Incidences of male rats with calculi and those with bladder tumors were strongly correlated, and chronic exposure of male rats to lower dietary concentrations of biphenyl (500 and 1,500 ppm) did not increase urinary pH and did not cause calculi formation, transitional cell hyperplasia, or bladder tumor development. There were relatively strong associations between incidences of rats with calculi and those with transitional cell hyperplasia and between incidences of rats with transitional cell hyperplasia and bladder tumors. In contrast, high concentrations of biphenyl in the diet of female rats had no effect on urinary pH, caused a much lower prevalence of urinary bladder calculi of a different composition, and resulted in no urinary bladder tumors. The urinary bladder calculi in the male rats were mainly composed of the conjugated biphenyl metabolite, potassium 4-hydroxybiphenyl-O-sulphate, whereas those of the female rats were predominantly composed of 4-hydroxybiphenyl and potassium bisulphate (which are hydrolysis products of potassium 4-hydroxybiphenyl-O-sulphate). There was no evidence of urinary bladder calculi formation or tumor development in male and female mice exposed to similar dietary concentrations of biphenyl. Results of a tumor initiation-promotion study in male rats support the proposal that biphenyl-induced sustained cell proliferation promotes initiated tumor cells in the urinary bladder. Finally, results of genotoxicity tests with biphenyl are predominantly negative or equivocal at best. The preponderance of evidence supports a mode of action for biphenyl in male rats only involving urinary tract calculi formation, urinary epithelium damage, sustained regenerative cell proliferation and hyperplasia, and subsequent bladder tumor formation. There is evidence that 2,5-dihydroxybiphenyl can undergo redox cycling to produce ROS that may damage DNA leading to tumor-initiating mutations, but it was not detected in urine of rats exposed to oral doses of 100 mg biphenyl/kg

and its metabolic precursor, 2-hydroxybiphenyl, is a minor urinary metabolite of biphenyl in rats (Meyer and Scheline, 1976).

Relevance of the hypothesized mode of action to humans. Although there are no studies in humans examining possible associations of biphenyl exposure with urinary bladder calculi formation or cancer, urinary bladder calculi have been reported in humans following exposure to other chemicals (Capen et al., 1999; Cohen, 1998, 1995). Urinary bladder calculi are, in general, expected to be irritating and lead to reparative cell proliferation regardless of composition or species; however, based on the anatomy of the urinary tract in humans and their upright, bipedal stature, calculi are either quickly excreted in urine or cause obstruction leading to pain and subsequent therapeutic removal of the calculi (Cohen, 1998, 1995). In contrast, the rodent horizontal quadruped stature is expected to promote calculi residency time in the bladder without causing obstruction (Cohen, 1998, 1995). In white populations, 95% of bladder tumors are transitional cell carcinomas such as those found in male rats exposed to high concentrations of biphenyl. Four case-control studies of urinary bladder cancer in white human populations found RRs for an association between a history of urinary tract stones and bladder carcinomas ranging from about 1.0 to 2.5 (Capen et al., 1999). Thus, the proposed mode of action is expected to be relevant to humans at exposure levels sufficient to cause urinary bladder calculi in humans, because: (1) calculi resulting from human exposure to other substances have been associated with urinary bladder irritation, regeneration, and cancer (Capen et al., 1999; Cohen, 1998, 1995) and (2) sulphate conjugation of hydroxylated biphenyl metabolites has been demonstrated in human tissues (see Section 3.3).

The underlying physiological factors determining the precipitation of 4-hydroxybiphenyl-O-sulphate in urine to form calculi in male rats, but not female rats, exposed to high dietary biphenyl concentrations are unknown. Elevated urine pH appears to play a role in the induction of urinary bladder tumors by biphenyl in the male rat (Umeda et al., 2002). Because humans on average have a slightly more acidic urine than the rat (Cohen, 1995), it is possible that humans might be less susceptible than the rat to the development of urinary bladder calculi. Given the lack of understanding of physiological factors that influence susceptibility in rats and the absence of specific human data on biphenyl-induced calculi or urinary stones, there is uncertainty in extrapolation of the dose-response relationship for biphenyl-induced calculi formation in male rats to humans.

Populations or lifestages particularly susceptible to the hypothesized mode of action. IARC (1999) noted that increased risks for bladder carcinoma in humans have been associated with cigarette smoking, exposure to infectious agents, such as *Shistosoma haematobium*, causing urinary tract inflammation, and a history for urinary tract infections in general. As such, people with these types of exposure or history may be particularly susceptible to the formation of

urinary calculi and urinary bladder cancer, but evidence supporting this inference is lacking. In addition, there are conditions (bladder diverticuli, neurogenic bladder, and staghorn renal pelvic calculi) that can increase the residency time of calculi in humans; thus, individuals with these conditions may also be particularly susceptible to biphenyl-induced bladder tumors under the hypothesized mode of action.

4.7.3.2. *Mode-of-Action Information for Liver Tumors in Female Mice*

Evidence that chronic oral exposure to biphenyl can cause liver tumors comes from the 2-year BDF_1 mouse bioassay by Umeda et al. (2005). Exposure to 2,000 or 6,000 ppm biphenyl in the diet, but not to 667 ppm, produced increased incidences of hepatocellular adenomas or carcinomas in female mice, but no carcinogenic response in male BDF_1 mice. Earlier studies found no carcinogenic response in $B6C3F_1$ or $B6AkF_1$ mice exposed to 517 ppm biphenyl in the diet for 18 months (Innes et al., 1969; NCI, 1968) or in ddY female mice exposed to 5,000 ppm biphenyl in the diet for 2 years (Imai et al., 1983). The only investigations into the mode of action for biphenyl-induced liver tumors in mice involve examinations of indicators of peroxisome proliferation following biphenyl exposure (Umeda et al., 2004b; Sunouchi et al., 1999). Thus, a mode of action involving PPARs is proposed and an evaluation of the supporting data follows.

4.7.3.2.1. *Hypothesized mode of action for liver tumors in female mice.* Proliferation of peroxisomes is regulated by a class of ligand-activated transcription factors known as PPARs. PPARα regulates induction of the peroxisome proliferation response in rodents and is thought to mediate at least some of the responses for hepatocarcinogens, including initiation of cellular events leading to transformation. Peroxisome proliferators (PPARα agonists) are a structurally diverse group of non- or weakly mutagenic chemicals that induce a suite of responses including the induction of tumors in rats and mice (Klaunig et al., 2003).

Klaunig et al. (2003) have proposed a mode of action for PPARα agonists involving the following key events. PPARα agonists activate PPARα to transcribe genes involved in peroxisome proliferation, cell cycling/apoptosis, and lipid metabolism. The changes in gene expression lead to changes in cell proliferation and apoptosis, and to peroxisome proliferation. Suppression of apoptosis coupled with increased cell proliferation allows transformed cells to persist and proliferate, resulting in preneoplastic hepatic foci and ultimately promotion of tumor growth via selective clonal expansion. Peroxisome proliferation may lead to oxidative stress, which potentially contributes to the proposed mode of action by causing indirect DNA damage and/or by causing cytotoxicity leading to reparative cell proliferation. PPARα agonists also inhibit gap junction intercellular communication and stimulate non-parenchymal hepatic Kupffer cells; these events are also thought to stimulate cell proliferation. Increases in the size and number of peroxisomes and induction of peroxisome-related gene expression (e.g., palmitoyl-

CoA oxidase and acyl-CoA oxidase) are regarded as indicators that the PPARα agonism mode of action is operative.

4.7.3.2.2. *Experimental support for the hypothesized mode of action for liver tumors in female mice*

Strength, consistency, and specificity of association, including support for the hypothesized mode of action in mice. Support for a possible association between biphenyl-induced proliferation of peroxisomes and liver tumor is limited to findings in female BDF_1 mice (which developed liver tumors following dietary exposure to 2,000 or 6,000 ppm); male BDF_1 mice did not develop liver tumors following exposure to concentrations as high as 6,000 ppm biphenyl. Dietary exposure of female BDF_1 mice to 16,000 ppm biphenyl for 13 weeks induced hepatocellular peroxisomes as evidenced by light microscopy detection of enlarged hepatocytes filled with eosinophilic fine granules and electron microscopy confirmation that the granules corresponded to increased numbers of peroxisomes (Umeda et al., 2004b). Significantly increased activities were measured for potassium cyanide-insensitive palmitoyl CoA oxidation in liver homogenate (up to 1.9-fold) and lauric acid 12-hydroxylation in liver microsomes (up to 3.8-fold) from female BDF_1 mice given oral doses up to 5.2 mmol/kg-day (800 mg/kg-day) for 3 days (Sunouchi et al., 1999).

The available data do not demonstrate strong, consistent, or specific associations between key events in the proposed mode of action and the development of liver tumors in female mice exposed to biphenyl. Klaunig et al. (2003) proposed that an adequate data set to support a PPARα agonism mode of action should meet the following criteria, most of which as noted in parentheses have not been investigated for biphenyl or its metabolites: (1) activation of PPARα (no data), (2) expression of peroxisomal genes including PPARα-mediated expression of cell cycle, growth, and apoptosis, and nonperoxisomal lipid gene expression (no data), (3) peroxisomal proliferation (limited data for biphenyl in mice as summarized in previous paragraph) and perturbation of cell proliferation and apoptosis (no data for mouse liver), (4) inhibition of gap junction intercellular communication (no data), (5) hepatocyte oxidative stress (no data), (6) Kupffer cell-mediated events (no data), and (7) selective clonal expansion (no data).

Dose-response concordance. The available data do not show concordance between the dose-response relationships for liver tumors in female BDF_1 mice exposed for 2 years to biphenyl in the diet (liver tumors at 2,000 or 6,000 ppm, but not 667 ppm) (Umeda et al., 2005) and liver peroxisome proliferation, the only key event in the proposed mode of action that has been investigated. Umeda (2004b) reported that, compared with controls, increased liver peroxisomes

were detected in female BDF_1 mice exposed to 16,000 ppm biphenyl in the diet for 13 weeks, but not in mice exposed to 500, 2,000, 4,000, 8,000, or 10,000 ppm.

Temporal relationship. Indicators of liver peroxisome proliferation were elevated in female mice, but not male mice, with oral exposure durations of 3 days following exposure to 800 mg/kg-day (increased activities of potassium cyanide-insensitive palmitoyl CoA oxidation and lauric acid 12-hydroxylation) (Sunouchi et al., 1999) and 13 weeks following exposure to 16,000 ppm in the diet (increased numbers of liver peroxisomes), but not at lower dietary concentrations (Umeda et al., 2004b).

Biological plausibility and coherence. The data are inadequate to evaluate the biological plausibility and coherence of the proposed mode of action as it relates to liver tumors in female mice exposed to biphenyl.

4.7.3.2.3. ***Other possible modes of action for liver tumors in mice.*** As discussed in Section 4.5.6, the overall weight of evidence from short-term standard genotoxicity tests with biphenyl and 4-hydroxybiphenyl is predominantly negative. A genotoxic mode of action for biphenyl-induced liver tumors in mice could be proposed based on the large metabolic capacity of the mouse liver to convert biphenyl to hydroxylated metabolites and evidence that metabolites of 2-hydroxybiphenyl (2,5-dihydroxybiphenyl and 2,5'-benzoquinone) can produce DNA damage (Tani et al., 2007; Balakrishnan et al., 2002; Sasaki et al., 2002; Sasaki et al., 1997; Pathak and Roy, 1993; Morimoto et al., 1989). However, hydroxylation of biphenyl to produce 2-hydroxybiphenyl appears to be a minor metabolic pathway in mice administered single i.p. doses of 30 mg biphenyl/kg (Halpaap-Wood et al., 1981b), and the available data are inadequate to establish that this genotoxic mode of action operates in the biphenyl induction of liver tumors in mice. There have been no in vitro or in vivo investigations of biphenyl-induced DNA adducts or ROS generation in mouse liver cells or of possible gender differences in the production of biphenyl-induced DNA adducts or other genotoxic events. Current mode-of-action information is inadequate to provide plausible explanations for why female BDF_1 mice exposed to high dietary concentrations of biphenyl develop liver tumors, but male BDF_1 mice exposed to 6,000 ppm and female ddY mice exposed to 5,000 ppm do not (Umeda et al., 2005; Imai et al., 1983).

4.7.3.2.4. ***Conclusions about the hypothesized mode of action for liver tumors in mice.***

A PPARα agonism mode of action for liver tumors in female mice exposed to 2,000 or 4,000 ppm biphenyl in the diet for 2 years is not adequately supported by the experimental data. This is based on the lack of concordance between dose-response relationships for biphenyl-induced liver tumors and proliferation of hepatocellular peroxisomes in female mice. Evidence

for increased hepatocellular peroxisomes in female mice was only found with 13-week exposure to 16,000 ppm biphenyl and not at several concentrations ≤10,000 ppm (Umeda et al., 2004b). Furthermore, a series of key events demonstrating PPARα agonism mode of action have not been identified.

Available data are inadequate to support alternative modes of action that propose direct or indirect genotoxic events from reactive biphenyl metabolites or ROS, respectively, as key events. Results from standard short-term genotoxicity tests are mostly negative or equivocal for biphenyl and 4-hydroxybiphenyl. Although there is some evidence for DNA damage from ROS generated from redox cycling between 2,5-dihydroxybiphenyl and phenylbenzoquinone, there are no investigations into the metabolic formation of 2-hydroxybiphenyl, 2,5-dihydroxybiphenyl, and phenylbenzoquinone in livers of biphenyl-exposed mice exposed to a range of biphenyl doses, no in vitro or in vivo investigations of biphenyl-induced DNA adducts or ROS generation in mouse liver cells, and no investigations of possible gender differences in capability to produce biphenyl-induced DNA adducts or other genotoxic events.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No specific information was identified that would specifically suggest an early childhood susceptibility for biphenyl toxicity. However, the developmental profiles of superoxide dismutase and catalase in humans that were reported by McElroy et al. (1992) indicate that the activities of both enzymes may be comparatively low before and at birth, placing humans in the perinatal period at an increased risk of adverse effects elicited by quinoid metabolites of biphenyl. Specifically, Buonocore et al. (2001) drew attention to the fact that the human brain has relatively low superoxide dismutase activity at birth. Given the limited data on age-specific ROS scavenging enzymes, any suggestions of childhood susceptibility to biphenyl is speculative.

Studies in animals provide evidence that biphenyl metabolism is mediated by CYP1A2 and CYP3A4 (Haugen, 1981). Phase II enzymes, such as sulphotransferases (SULTs) and UGTs, may be involved in conjugation activities with hydroxybiphenyls in mammalian tissues (Pacifici et al., 1991; Bock et al., 1980). CYP1A2 expression is negligible in the early neonatal period, but is significantly increased to 50% of adult levels by 1 year of age (Sonnier and Cresteil, 1998). In general, SULTs and UGTs, depending on the isoforms, also exhibit differential expression during human development (Duanmu et al., 2006; Strassburg et al., 2002). To the extent that metabolism increases or reduces the toxicity of biphenyl, changes in the expression of Phase I and II enzymes during development can influence susceptibility to biphenyl toxicity. Specific isoforms of CYPs and Phase II enzymes have not been identified as the principal catalyzers involved in biphenyl metabolism and the effect of differences in enzyme expression on childhood susceptibility to biphenyl has not been established.

4.8.2. Possible Gender Differences

Benford and Bridges (1983) evaluated the sex- and tissue-specific induction of biphenyl 2-, 3-, and 4-hydroxylase activities in microsomal preparations or primary hepatocyte cultures from male and female Wistar rats. No differences in biphenyl hydroxylase activities were observed between the sexes. However, there were some sex differences in the way tissues responded to the action of enzyme inducers. For example, the CYP1A inducer α-naphthoflavone strongly induced 2-hydroxylase in male liver but had no effect on female liver. Betamethasone induced 2-hydroxylase activity in female liver but inhibited it in male liver. The available limited human data do not suggest that gender differences exist in the response to biphenyl exposure. However, available animal data suggest gender-related differences in susceptibility to tumors (i.e., bladder tumors in male, but not female, F344 rats and increased incidences of liver tumors in female, but not male, BDF_1 mice administered biphenyl in the diet for a lifetime).

4.8.3. Other

The limited information on the specifics of biphenyl metabolism and toxic effects in humans does not allow a meaningful assessment of populations that might be highly susceptible to the adverse effects of biphenyl. For example, there is as yet no clear attribution of CYP isozymes to the various biphenyl hydroxylases and no information on which sulphotransferases and glucuronidases conjugate hydroxylated biphenyl metabolites. It is known that many CYP isozymes, as well as glucuronidases, exist in polymorphic forms with catalytic activities that differ from the wild type. In addition, such enzyme polymorphisms display specific distributions across populations and ethnicities that might put some at increased risk and others at decreased risk of adversity from biphenyl exposure. This lack of information represents a data gap.

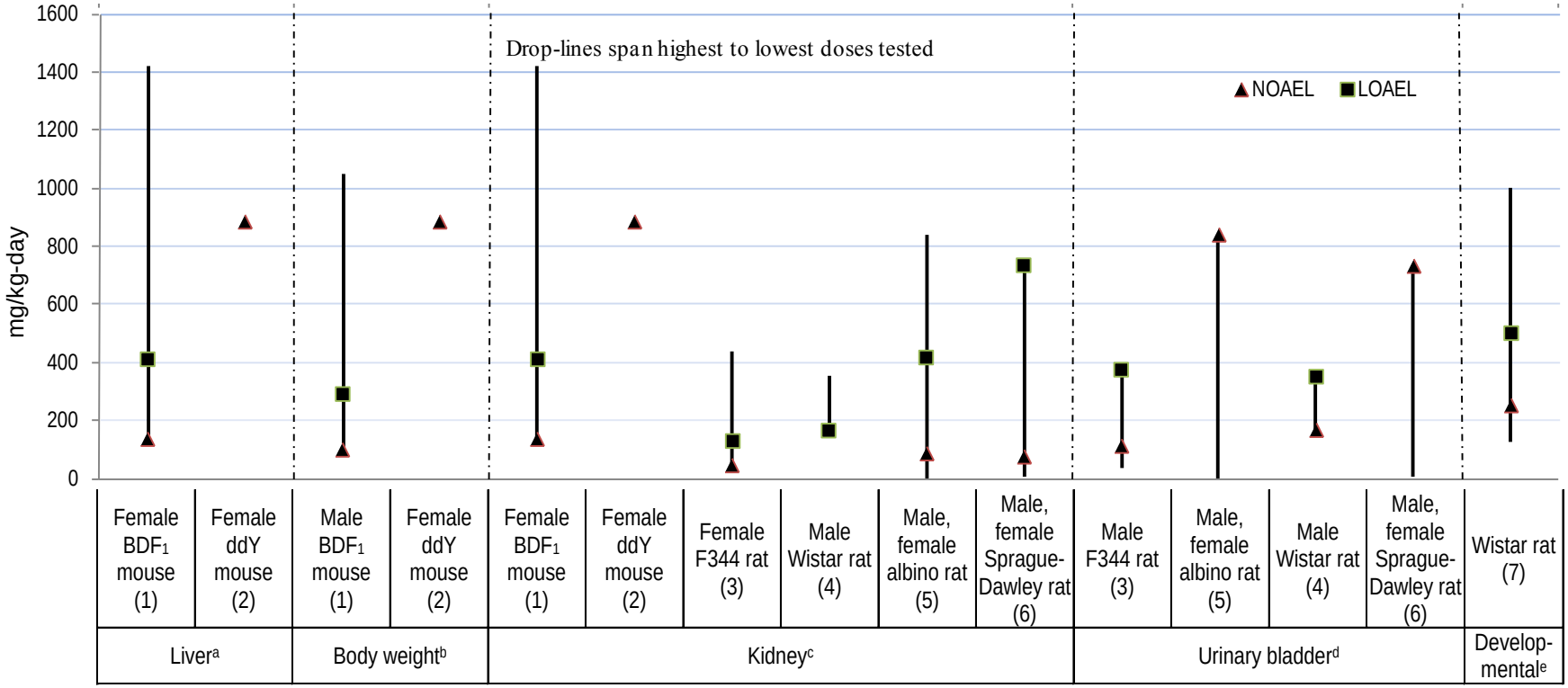
5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Candidate Principal Studies and Candidate Critical Effects—with Rationale and Justification

No information was located regarding possible associations between oral exposure to biphenyl and health outcomes in humans.

As discussed in Section 4.6.1, the most sensitive targets of toxicity following oral exposure to biphenyl are the liver, urinary system, body weight, and developing organism (see Figure 5-1). In the rat, chronic oral studies identified the kidney and urinary bladder as critical noncancer targets (see Figure 5-1 for LOAELs and NOAELs found in these studies). Kidney effects observed include: renal pelvis transitional cell hyperplasia and hemosiderin deposits in female F344 rats at doses ≥128 mg/kg-day and renal pelvis mineralization at 378 mg/kg-day (Umeda et al., 2002); kidney stone formation and obstructive pyelonephritis with tubular atrophy, tubular cysts, and fibrosis in male and female Wistar rats at 165 and 370 mg/kg-day, respectively (Shiraiwa et al., 1989); renal lymphocytic infiltration, tubular atrophy, and tubular cysts in male and female albino rats at doses ≥420 mg/kg-day (Ambrose et al., 1960); mild renal tubular degeneration in male albino rats at 250 or 450 mg/kg-day (Pecchiai and Saffiotti, 1957; not plotted in Figure 5-1 because quantitative data were not included in the study report); and renal tubular dilatation in male and female Sprague-Dawley rats at 732 mg/kg-day (Dow Chemical Co, 1953). An increased incidence of urinary bladder hyperplasia associated with calculi or "stones" was observed in male and female F344 rats at 378 and 438 mg/kg-day, but not at 110 and 128 mg/kg-day, respectively (Umeda et al., 2002). Elevated incidences of the same lesion were observed in male and female Wistar rats at 353 and 370 mg/kg-day, respectively (Shiraiwa et al., 1989). In contrast, urinary bladder hyperplasia and calculi were not observed in male or female albino rats at doses as high as 840 mg/kg-day (Ambrose et al., 1960) or in male or female Sprague-Dawley rats exposed to doses as high as 732 mg/kg-day (Dow Chemical Co, 1953).



[a]Increased plasma liver enzymes in BDF_1 mice.
[b]Decreased body weight (>10% lower than controls) in BDF_1 mice.
[c]Increased incidences of kidney lesions including: mineralization in outer medulla in BDF_1 mice; renal pelvis transitional cell hyperplasia and hemosiderin deposits in F344 rats; kidney stone formation in Wistar rats; renal tubular atrophy in albino rats; renal tubular dilatation in Sprague-Dawley rats.
[d]Increased incidences of urinary bladder calculi or stones and hyperplasia in F344 rats and Wistar rats.
[e]Increased number of litters with fetal skeletal anomalies in Wistar rats.

(1) = Umeda et al., 2005; (2) = Imai et al., 1983; (3) = Umeda et al., 2002; (4) = Shiraiwa et al., 1989; (5) = Ambrose et al., 1960; (6) = Dow Chemical Co., 1953; (7) = Khera et al., 1979

Figure 5-1. NOAELs and LOAELs for noncancer effects in rats and mice from repeated oral exposure to biphenyl.

In mice, chronic oral toxicity studies identified the liver, kidney, and body weight as critical noncancer targets (see Figure 5-1 for NOAELs and LOAELs for these effects). In BDF_1 mice, significantly ($p < 0.05$) increased plasma levels of enzymes indicative of liver damage were observed at dose levels of 1,050 mg/kg-day in males and ≥414 mg/kg-day in females (Umeda et al., 2005), but no exposure-related changes in liver enzymes were observed in female ddY mice at 885 mg/kg-day (Imai et al., 1983). Significantly increased incidence of mineralization of the renal outer medulla and increased BUN levels were observed in BDF_1 mice at ≥219 mg/kg-day (males) and ≥414 mg/kg-day (females), respectively (Umeda et al., 2005), but exposure-related histological changes in the kidney were not found in female ddY mice at 885 mg/kg-day (Imai et al., 1983). Following the same pattern of apparent strain difference in susceptibility to biphenyl toxicity, body weights were decreased by >10% at≥291 mg/kg -day in male BDF_1 mice and ≥414 mg/kg-day in females (Umeda et al., 2005), but body weights in female ddY mice exposed to 885 mg/kg-day were similar to control values (Imai et al., 1983). Shorter-duration oral exposure (13 weeks) of mice to biphenyl at higher dietary concentrations (estimated doses ≥1,500 mg/kg-day) has also been shown to affect body and/or liver weights in mice (Umeda et al., 2004b).

In the only available oral developmental toxicity study (Khera et al., 1979), frank maternal toxicity (increased mortality [5/20 versus 0/18 in controls] and decreased number of dams with live fetuses [9/20 versus 16/18 in controls]) occurred at the highest dose (1,000 mg/kg-day). Significantly increased incidences of fetuses with skeletal anomalies were noted at doses ≥500 mg/kg-day. The NOAEL and LOAEL of 250 and 500 mg/kg-day for delayed skeletal development are noted in Figure 5-1.

The 2-year dietary studies in F344 rats (Umeda et al., 2002) and BDF_1 mice (Umeda et al., 2005) and the developmental study in Wistar rats (Khera et al., 1979) were selected as candidate principal studies for deriving the RfD because they provide the best available data (adequate number of dose groups and dose spacing, sufficient group sizes, comprehensive endpoint assessment and quantitation of results) to describe dose-response relationships for the critical effects in rats and mice associated with chronic or gestational exposure to biphenyl.

Candidate critical effects from the chronic study in F344 rats (Umeda et al., 2002) were: (1) nodular or simple transitional cell hyperplasia in the renal pelvis of males and females, (2) mineralization in the renal pelvis or renal papillary mineralization in males and females, (3) renal hemosiderin deposits in females, and (4) transitional cell hyperplasia in the urinary bladder of males. Candidate critical effects from the chronic study in BDF_1 mice (Umeda et al., 2005) were: (1) decreased body weight in males and females, (2) mineralization of the renal inner stripe-outer medulla in males and females, (3) BUN in males and females, and (4) serum liver enzyme activities (AST [GOT], ALT [GPT], AP, and LDH) in females. The candidate

critical effect from the rat oral developmental toxicity study (Khera et al., 1979) was litters with fetal skeletal anomalies from Wistar rat dams exposed during gestation.

5.1.2. Methods of Analysis—Including Models (e.g., PBPK, BMD)

Datasets modeled included selected nonneoplastic lesions in the urinary system of male and female F344 rats (Table 5-1) exposed to biphenyl in the diet for 2 years (Umeda et al., 2002), mineralization in the kidney of male and female BDF_1 mice (Table 5-2) exposed to biphenyl in the diet for 2 years (Umeda et al., 2005), and litters with skeletal anomalies from Wistar rat dams (Table 5-3) administered biphenyl by gavage on GDs 6–15 (Khera et al., 1979).

Table 5-1. Datasets employed in the BMD modeling of nonneoplastic effects in the urinary tract of male and female F344 rats exposed to biphenyl in the diet for 2 years

	Males (n = 50)				Females (n = 50)			
Biphenyl dietary concentration (ppm)	**0**	**500**	**1,500**	**4,500**	**0**	**500**	**1,500**	**4,500**
TWA body weight (kg)[a]	**0.411**	**0.412**	**0.408**	**0.357**	**0.251**	**0.246**	**0.246**	**0.216**
Calculated dose (mg/kg-d)[b]	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Effect								
Renal pelvis								
Nodular transitional cell hyperplasia	0	1	1	21[c]	0	0	1	12[c]
Simple transitional cell hyperplasia	6	8	5	19[d]	3	5	12[d]	25[c]
Mineralization	9	6	10	18[e]	12	12	18	27[d]
Other kidney effects								
Hemosiderin deposit[f]	0	0	0	0	4	8	22[c]	25[c]
Papillary mineralization	9	9	14	23[d]	2	6	3	12[c]
Bladder								
Combined transitional cell hyperplasia[g]	0	0	0	45	1	0	1	10

[a]TWA body weight calculated using graphically-presented body weight data in the study report of Umeda et al. (2002).
[b]Calculated doses based on calculated TWA body weights and chronic reference food consumption values for F344 rats (0.030 and 0.021 kg/day for males and females, respectively; taken from Table 1-6 of U.S. EPA, 1988).
[c]Significantly different from control group ($p < 0.01$) according to χ^2 test.
[d]Significantly different from control group ($p < 0.05$) according to χ^2 test.
[e]Significantly different from controls ($p < 0.05$) according to Fisher's exact test.
[f]Male data for incidences of hemosiderin deposits not selected for quantitative analysis.
[g]Female data for incidences of combined transitional cell hyperplasia not selected for quantitative analysis.

Source: Umeda et al. (2002).

Table 5-2. Datasets employed in the BMD modeling of body weight, selected clinical chemistry results, and histopathological kidney effects in male and female BDF_1 mice exposed to biphenyl in the diet for 2 years

Endpoint	Biphenyl concentration in the diet (ppm)			
	0	**667**	**2,000**	**6,000**
Males				
Dose (mg/kg-d)	**0**	**97**	**291**	**1,050**
Histopathological kidney effect	n = 50	n = 49	n = 50	n = 50
Mineralization inner stripe-outer medulla	9	8	14	14
Clinical chemistry parameter	n = 34	n = 39	n = 37	n = 37
BUN (mg/dL)	20.2 ± 3.6	22.0 ± 4.0	23.2 ± 4.4[a]	22.9 ± 2.7[b]
Body weight	n = 35	n = 41	n = 41	n = 39
Mean terminal body weight (g)	46.9 ± 4.9	43.1 ± 7.9	42.9 ± 6.0[a]	32.4 ± 3.6[b]
Females				
Dose (mg/kg-d)	**0**	**134**	**414**	**1,420**
Histopathological kidney effect	n = 50	n = 50	n = 50	n = 49
Mineralization inner stripe-outer medulla	3	5	12[c]	26[d]
Clinical chemistry parameter	n = 28	n = 20	n = 22	n = 31
AST (IU/L)	75 ± 27	120 ± 110	211 ± 373[b]	325 ± 448[b]
ALT (IU/L)	32 ± 18	56 ± 46	134 ± 231[b]	206 ± 280[b]
AP (IU/L)	242 ± 90	256 ± 121	428 ± 499	556 ± 228[b]
LDH (IU/L)	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161[a]
BUN (mg/dL)	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7[b]
Body weight	n = 31	n = 22	n = 25	n = 32
Mean terminal body weight (g)	34.0 ± 4.0	32.5 ± 3.3	30.5 ± 3.1[b]	25.5 ± 3.0[b]

[a]Significantly different from controls ($p < 0.05$) according to Dunnett's test.
[b]Significantly different from controls ($p < 0.01$) according to Dunnett's test.
[c]Significantly different from controls ($p < 0.05$) according to Fisher's exact test.
[d]Significantly different from controls ($p < 0.01$) according to Fisher's exact test.

Source: Umeda et al. (2005).

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Table 5-3. BMD modeling dataset for incidence of litters with fetal skeletal anomalies from Wistar rat dams administered biphenyl by gavage on GDs 6–15

Effect	Dose (mg/kg-d)				
	0	125	250	500	1,000
Litters with fetal skeletal anomalies[a]/litters examined	8/16	11/20	13/18	15[b]/18	6/9

[a]The study authors reported one runted fetus in the control group and one fetus with kinky tail in the 250 mg/kg-day dose group, which may have influenced the reported incidence data for anomalous litters/litters examined.
[b]Significantly different from controls ($p < 0.05$) according to Fisher's exact test conducted for this review.

Source: Khera et al. (1979).

Consistent with the EPA's draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000a), dose-response modeling was conducted using the U.S. EPA's benchmark dose (BMD) software (BMDS, version 2.1.2.) to calculate potential points of departure (PODs) for deriving the RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$).

All available dichotomous models in the EPA Benchmark Dose (BMD) Software (BMDS) (version 2.1.2) were fit to the incidence data for each dataset. The multistage model was run for all polynomial degrees up to n-1 (where n is the number of dose groups including control). Adequate model fit was judged by three criteria: chi-square goodness-of-fit *p*-value ($p \geq 0.1$), visual inspection of the fit of the dose-response curve to the data points, and a value of <2 for the largest scaled residual for any data point in the dataset (including the control). Among all of the models providing adequate fit to the data, the lowest BMDL (95% lower confidence limit on the BMD) was selected as the potential point of departure (POD) when the difference between the BMDLs estimated from these models was more than threefold; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) was chosen as the candidate POD. BMDs and BMDLs associated with an extra risk of 10% were calculated for all models. In the absence of information to identify a biologically significant level of response for an endpoint, a benchmark response (BMR) of 10% extra risk is typically chosen as an appropriate response level for dichotomous data. A BMR of 10% is also recommended to facilitate a consistent basis of comparison across assessments.

A BMR of 10% extra risk was selected to derive the POD for developmental effects from the Khera et al. (1979) study because the endpoint was characterized as affected litters. A BMR of 5% extra risk has typically been used for reproductive and developmental studies when data are reported as affected pups within litters (U.S. EPA, 2000a). Since this level of reporting was not available in Khera et al. (1979), nested models could not be used. Thus, a BMR of 10% extra risk among affected litters was employed in order to better approximate a 5% extra risk in

affected offspring and to recognize the litter as the experimental unit. BMDs and BMDLs associated with extra risk of 5% for all endpoints were also calculated for comparison.

When standard models failed to provide adequate fit to the data, modifications of these standard models (i.e., parameter restriction adjustments, specification of initial parameter values, or use of alternative models) were attempted in an effort to achieve adequate fit. If these modifications failed to achieve adequate fit, the highest dose was dropped, and the entire modeling procedure was repeated. If an adequate fit could not be achieved after dropping the highest dose, then the dataset was determined to be unsuitable for BMD modeling.

For continuous data, all continuous models available in the EPA BMDS (version 2.1.2) were first applied to the data while assuming constant variance. If the data were consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of all the continuous models to the mean were evaluated while assuming constant variance. In the absence of information to indicate a biologically significant level of response, BMDs and BMDLs were calculated based on a BMR representing a change of 1 SD from the control. BMDs and BMDLs for decreased body weight were also calculated for a BMR of 10% decrease from the control (i.e., 10% relative deviation [RD]) because a 10% decrease in body weight is generally considered to represent a minimally biologically significant effect. For serum enzyme activities (AST, ALT, AP, LDH), BMDs and BMDLs were also calculated for a BMR of 100% increase from the control (i.e., twofold or 1 RD; BMD_{1RD} and $BMDL_{1RD}$). Several expert organizations, particularly those concerned with early signs of drug-induced hepatotoxicity, have identified an increase in liver enzymes (AST, ALT, AP) compared with concurrent controls of two- to fivefold as an indicator of concern for hepatic injury (EMEA, 2006; Boone et al., 2005). Because LDH, like liver enzymes, is one of the more specific indicators of hepatocellular damage in most animal species and generally parallels changes in liver enzymes in toxicity studies where liver injury occurs, a similar twofold increase in LDH is considered indicative of liver injury in experimental animals. A similar approach was taken for BUN.

Adequate model fit was judged by three criteria: goodness-of-fit *p*-value ($p \geq 0.1$), visual inspection of the dose-response curve, and a value of <2 for the largest scaled residual for any data point in the data set (including the control). Among all of the models providing adequate fit to the data, the lowest BMDL was selected as the potential POD when the BMDLs estimated from these models varied by more than threefold; otherwise, the BMDL from the model with the lowest AIC was chosen as the candidate POD. When the test for constant variance failed, all models were run again while applying the power model integrated into the BMDS to account for nonhomogeneous variance. When the nonhomogeneous variance model provided an adequate fit ($p \geq 0.1$) to the variance data, the models were evaluated using the nonhomogeneous variance model. Model fit and POD selection proceeded as described earlier. When both tests for variance (constant and nonhomogeneous) provided inadequate fit to the variance data, model restriction adjustments were attempted in an effort to achieve adequate fit. If these adjustments

failed to achieve better fit, then the highest dose was dropped and the entire modeling procedure was repeated. If an adequate fit could not be achieved after dropping the highest dose, then the dataset was determined to be unsuitable for BMD modeling.

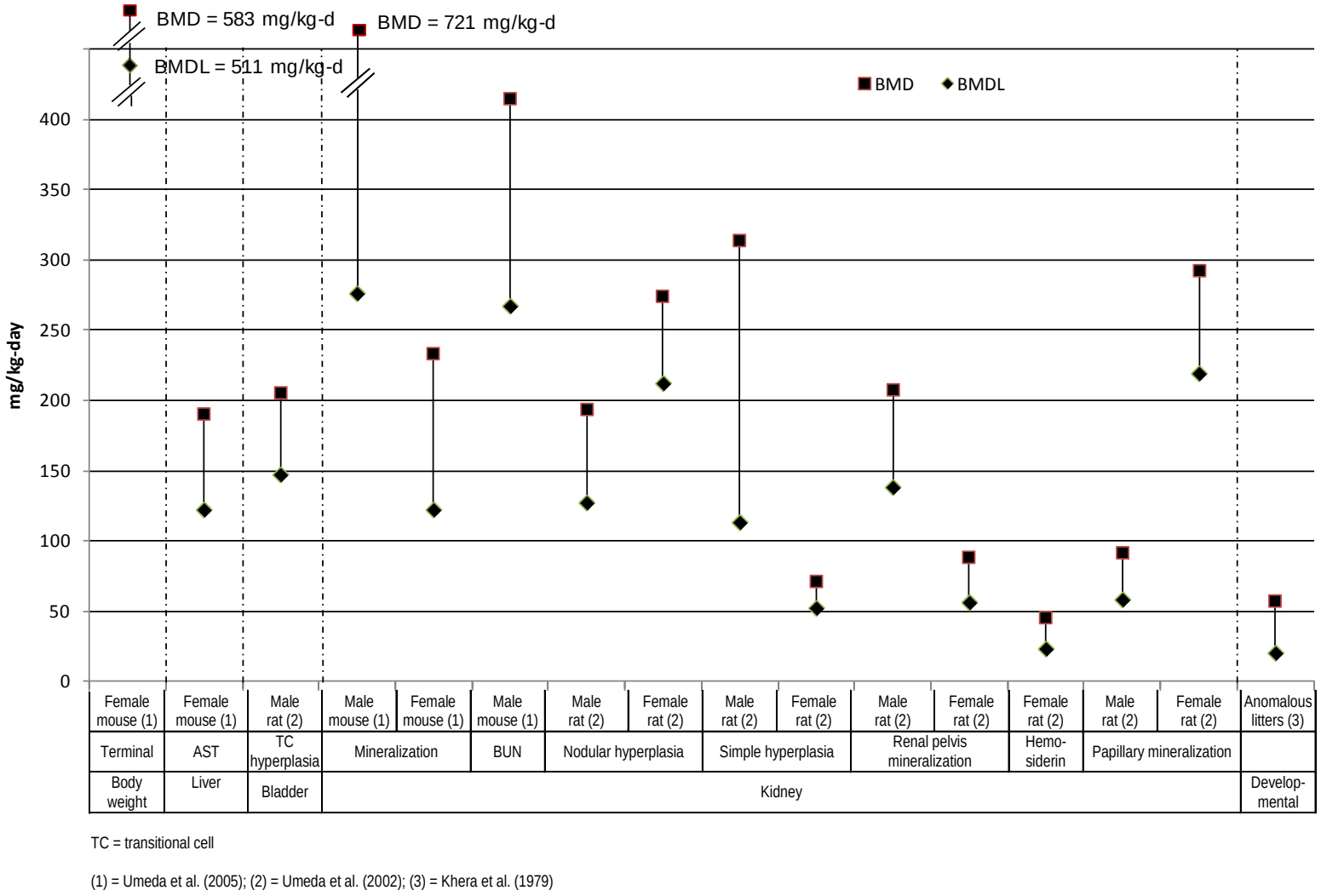
Summary modeling results are presented in Table 5-4 and Figure 5-2; more detailed modeling results are presented in Appendix C (Tables C-4 through C-24 and respective model output files). The BMDs and BMDLs shown in Table 5-4 and Figure 5-2 are those from the best-fitting models for each endpoint. BMDs and BMDLs for serum AST levels in female mice and for serum BUN levels in male mice were derived after dropping the data from the highest dose groups. For datasets to which no model could be fit, NOAELs and LOAELs were considered for candidate PODs.

Table 5-4. Summary of BMDs/BMDLs for selected nonneoplastic effects following oral exposure of rats and mice to biphenyl

	Males				Females			
	Best fitting model	BMR	Benchmark result (mg/kg-d)		Best fitting model	BMR	Benchmark result (mg/kg-d)	
			BMD	BMDL			BMD	BMDL
F344 rats (Umeda et al., 2002); biphenyl in the diet for 2 yrs								
Kidney								
Renal pelvis								
Transitional cell nodular hyperplasia	Multistage 3-degree	10%	193	127	Multistage 2-degree	10%	274	212
Transitional cell simple hyperplasia	Gamma	10%	314	113	Gamma	10%	71	52
Mineralization	Log-probit	10%	208	138	Multistage 1-degree	10%	88	56
Kidney – other								
Hemosiderin deposit	Not selected[b]	10%	–	–	Dichotomous-Hill	10%	45	23
Papillary mineralization	Multistage 1-degree	10%	92	58	Logistic	10%	292	219
Bladder								
Transitional cell hyperplasia	Gamma	10%	205	147	Not selected[b]	10%	–	–
BDF_1 mice (Umeda et al., 2005); biphenyl in the diet for 2 yrs								
Kidney								
Mineralization	Log-logistic	10%	721	276	Log-logistic	10%	233	122
Clinical chemistry								
AST	Not selected[b]	1RD	–	–	Power	1RD	190[a]	122[a]
ALT	Not selected[b]	1RD	–	–	No adequate fit[c]	1RD	–	–
LDH	Not selected[b]	1RD	–	–	No adequate fit[c]	1RD	–	–
AP	Not selected[b]	1RD	–	–	No adequate fit[c]	1RD	–	–
BUN	Linear	1SD	415[a]	267[a]	No adequate fit[c]	1SD	–	–
Body weight								
Terminal body weight	No adequate fit[c]	0.1RD	–	–	Linear	0.1RD	583	511
Wistar rats (Khera et al., 1979); biphenyl by gavage to dams on GDs 6–15								
Litters with fetal skeletal anomalies					Log-logistic	10%	57	20

[a]Adequate fit obtained only after excluding results from the highest dose group.
[b]"Not selected" indicates that the data set was not selected for dose-response analysis because either a treatment-related effect was not observed or because the response observed in the other sex in the same study was more robust.
[c]"No adequate fit" indicates that none of the models in BMDS provided an adequate fit to the data.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_{1RD}$ = 100% RD from control mean value; $_{0.1RD}$ = 10% RD from control mean value; $_{1SD}$ = 1 SD from control mean value)



TC = transitional cell

(1) = Umeda et al. (2005); (2) = Umeda et al. (2002); (3) = Khera et al. (1979)

Figure 5-2. BMDs and BMDLs for selected noncancer effects in rats and mice from repeated oral exposure to biphenyl.

Examination of the BMD and BMDL values in Table 5-4 and Figure 5-2 reveals BMD/BMDL pairs for four kidney effects and for the developmental effect that are clustered below BMD/BMDL pairs for the other effects. The BMDL values in this cluster range from 20 to 58 mg/kg-day and identify the following as the most sensitive nonneoplastic effects associated with repeated oral exposure to biphenyl in animals: (1) renal transitional cell hyperplasia (simple) in female F344 rats (52 mg/kg-day), (2) renal mineralization in female F344 rats (56 mg/kg-day), (3) renal hemosiderin deposition in female F344 rats (23 mg/kg-day), (4) renal papillary mineralization in male F344 rats (58 mg/kg-day), and (5) increased litters with fetal skeletal anomalies in Wistar rats (20 mg/kg-day).

NOAEL values for endpoints with datasets for which adequate model fits could not be obtained using BMDS were higher than the BMDL values for these five kidney and developmental endpoints. These include selected clinical chemistry parameters in female BDF_1 mice (NOAELs for LDH, AP, and BUN: 414 mg/kg-day; NOAEL for ALT: 134 mg/kg-day) and terminal body weight in male BDF_1 mice (NOAEL: 97 mg/kg-day).

The increased fetal skeletal anomalies in Wistar rats was selected as the critical effect for deriving an oral RfD because it was considered to be an adverse effect and resulted in the most sensitive POD ($BMDL_{10}$ of 20 mg/kg-day) observed compared with other PODs for biphenyl-induced kidney effects.

In EPA's guidance document entitled, *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011), the Agency endorses a hierarchy of approaches for converting doses administered orally to laboratory animal species to human equivalent oral exposures in deriving the RfD, with the preferred approach being physiologically-based toxicokinetic modeling. An alternate approach includes using chemical-specific information in the absence of a complete physiologically-based toxicokinetic model. In lieu of a toxicokinetic model or chemical-specific data to inform the generation of human equivalent oral exposures, EPA endorses body weight scaling to the ¾ power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from laboratory animals to humans for the purpose of deriving an RfD. When $BW^{3/4}$ scaling is used in deriving the RfD, EPA also advocates a reduction in the interspecies uncertainty factor from 10 to 3, as $BW^{3/4}$ scaling addresses predominantly toxicokinetic (and some toxicodynamic) aspects of the UF_A.

Statements in the guidance raise some important uncertainties in applying allometric scaling, and more specifically $BW^{3/4}$ scaling, when trying to extrapolate across different sized individuals within a species (e.g., between neonates and adults) or across individuals in different lifestages between species (e.g., between fetal rats and adult humans). Furthermore, the data on which to base a default allometric scaling factor for converting the administered dose in a laboratory animal in a different lifestage to a comparable dose in an adult human are sparse, and thus more uncertain. For these reasons, a $BW^{3/4}$ scaling factor has not been applied as a default

approach (in combination with a reduced default UF for interspecies extrapolation) when extrapolating from developmental effects in laboratory animals to adult humans when deriving the RfD.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

Consideration of available dose-response data led to the selection of the developmental study (Khera et al., 1979) and fetal skeletal anomalies in litters from biphenyl-treated pregnant Wistar rats as the principal study and critical effect, respectively, for RfD derivation. The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty resulting in a composite UF of 100. This composite uncertainty factor was applied to the selected POD to derive an RfD.

- An UF of 10 was applied to account for interspecies variability in extrapolation from laboratory animals (rats) to humans because information is not available to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans.

- An UF of 10 was applied to account for intraspecies variability in susceptibility to biphenyl, as quantitative information for evaluating toxicokinetic and toxicodynamic differences among humans are not available.

- An UF of 1 was applied for use of data from a subchronic study to assess potential effects from chronic exposure because the POD is from a developmental toxicity study. Consistent with EPA practice (U.S. EPA, 1991), an uncertainty factor was not applied to account for the extrapolation from less than chronic exposure because developmental toxicity resulting from a narrow period of exposure was used as the critical effect. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure.

- An UF of 1 was applied for extrapolation from a LOAEL to a NOAEL because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of 10% increase in incidence of litters with skeletal anomalies was selected under an assumption that it represents a minimal biologically significant change.

- An UF of 1 to account for database deficiencies was applied. The biphenyl database includes chronic toxicity studies in rats (Umeda et al., 2002; Shiraiwa et al., 1989; Ambrose et al., 1960; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953) and mice (Umeda et al., 2005; Imai et al., 1983); subchronic toxicity studies in rats (Shibata et al., 1989b; Shibata et al., 1989a; Kluwe, 1982; Søndergaard and Blom, 1979; Booth et al., 1961) and mice (Umeda et al., 2004b); a developmental toxicity study in rats (Khera et al., 1979); and one- and three-generation reproductive toxicity studies in rats (Ambrose et al., 1960; Dow Chemical Co, 1953). Epidemiological studies provide some evidence that biphenyl may induce functional changes in the nervous system at concentrations in

excess of occupational exposure limits. Seppäläinen and Häkkinen (1975) reported small increases in anomalies in nerve conduction, EEG, and ENMG signals in workers exposed to biphenyl during the production of biphenyl-impregnated paper at concentrations that exceeded the occupational limit by up to 100-fold, and Wastensson et al. (2006) reported a cluster of PD in a Swedish factory manufacturing biphenyl-impregnated paper. No other clusters of PD have been reported in biphenyl exposed populations, and Wastensson et al. (2006) acknowledged that chance is an alternative explanation for this cluster. Studies in experimental animal models have not identified effects on the nervous system following biphenyl exposure. Accordingly, these epidemiologic studies do not suggest that the nervous system is a sensitive target of biphenyl toxicity, and therefore, the lack of nervous system-specific studies is not considered a gap in the biphenyl toxicity database.

The RfD for biphenyl was calculated as follows:

$$\text{RfD} = \text{BMDL}_{10} \div \text{UF}$$
$$= 20 \text{ mg/kg-day} \div 100$$
$$= 0.2 \text{ mg/kg-day}$$

5.1.4. Previous RfD Assessment

The previous IRIS assessment for biphenyl, posted to the IRIS database in 1987, derived an oral RfD of 0.05 mg/kg-day based on kidney damage in albino rats administered biphenyl for 2 years at dietary levels ≥0.5% (Ambrose et al., 1960). U.S. EPA considered the dietary level of 0.1% (50 mg/kg-day using a food factor of 0.05/day) to represent a NOAEL due to the following: (1) uncertainty in the significance of effects observed at lower doses as compared to the more certain adverse effect level of 0.5% in the diet and (2) supportive findings of 0.1% biphenyl as a NOAEL in an unpublished report of a subchronic rat feeding study and a three-generation rat reproduction study performed by Stanford Research Institute (Dow Chemical Co, 1953). The NOAEL of 50 mg/kg-day was divided by a total UF of 1,000 (10 for extrapolation from animals to humans, 10 for protection of sensitive human subpopulations, and a modifying factor of 10 to account for intraspecies variability demonstrated in the threshold suggested by the data in the chronic animal study).

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Human data are limited to assessments of possible associations between occupational exposure to biphenyl and health outcomes where inhalation is presumed to have been the major exposure route. Clinical signs and abnormal electrophysiological test results among workers exposed to biphenyl during the production of biphenyl-impregnated fruit wrapping paper provide evidence of biphenyl-induced neurological effects (Seppalainen and Hakkinen, 1975; Häkkinen et al., 1973; Häkkinen et al., 1971). Case reports include an account of periodic loss of strength and eventual signs of chronic hepatitis in a woman during a 25-year period of employment at a

fruit-packing facility where biphenyl-impregnated paper was used (Carella and Bettolo, 1994) and a cluster of five cases of PD (0.9 cases expected) at a facility manufacturing biphenyl-impregnated paper (Wastensson et al., 2006). None of these studies provided air monitoring data adequate to characterize workplace exposures to biphenyl. Therefore, data from the available human studies could not be used for dose-response analysis and derivation of an RfC.

Limited information is available regarding the effects of inhaled biphenyl in laboratory animals. In mice, repeated airborne exposure to biphenyl (7 hours/day, 5 days/week for 2 weeks) at concentrations as high as 54.75 ppm (345.5 mg/m^3) appeared to cause no symptoms (Sun, 1977b). In a series of studies that included repeated inhalation exposure of rabbits, rats, and mice to air containing biphenyl for periods of 68–94 days (Deichmann et al., 1947; Monsanto, 1946), rabbits exhibited no signs of exposure-related adverse effects at concentrations as high as 300 mg/m^3. Irritation of mucous membranes was observed in rats at concentrations of 40 and 300 mg/m^3. Mice were the most sensitive to inhaled biphenyl; irritation of the upper respiratory tract was noted at a concentration of 5 mg/m^3 (Deichmann et al., 1947; Monsanto, 1946), but other biphenyl concentrations were not tested in this experiment. The limitations of a single exposure level and poorly reported study details preclude the use of this study for RfC derivation.

Repeated exposure of mice to biphenyl at vapor concentrations of 25 or 50 ppm (157.75 or 315.5 mg/m^3) for 13 weeks resulted in high incidences of pneumonia and tracheal hyperplasia, and high incidences of congestion and edema in the lungs, liver, and kidney (Sun, 1977a). The following study limitations and lack of supporting data preclude the usefulness of this study for deriving an RfC for biphenyl. Measured biphenyl exposure concentrations varied greatly during the first half of the 13-week exposure period; for example, in the high concentration group (target concentration of 50 ppm), the measured concentrations ranged from 5 to 102 ppm during the first 45 exposure sessions. High mortality after 46 exposures (as a result of accidental overheating of the chambers) necessitated the use of 46 replacement animals; these replacement animals received the same total number of exposure sessions as the surviving animals from the original groups, but exposures were not concurrent. Histopathological findings were reported only for males and females combined. Reports of lung congestion and hemorrhagic lungs in some control mice were not confirmed histopathologically, and congestion in the lung, liver, and kidney were considered by the study pathologist a likely effect of the anesthetic used for killing the mice. The severity of reported histopathologic lesions was not specified.

Given these deficiencies, the Sun Company Inc. (1977a) 13-week inhalation mouse study, the only available study that employed at least subchronic-duration exposure and multiple biphenyl exposure levels, is considered inadequate for RfC derivation. An RfC was not derived due to the significant uncertainty associated with the inhalation database for biphenyl, and route-to-route extrapolation was not supported in the absence of a physiologically based

pharmacokinetic (PBPK) model. Although an RfC cannot be derived, it should be noted that the available inhalation data provides some evidence that inhalation exposure to biphenyl could induce respiratory or systemic lesions.

5.2.2. Previous RfC Assessment

No RfC was derived in the previous (1985) IRIS assessment.

5.3. UNCERTAINTIES IN THE RfD AND RfC

Risk assessments should include a discussion of uncertainties associated with the derived toxicity values. To derive the oral RfD, the UF approach (U.S. EPA, 2002, 1994b) was applied to a POD of 20 mg/kg-day (see Section 5.1). Factors were applied to the POD to account for extrapolating from responses observed in an animal bioassay to a diverse human population of varying susceptibilities. Uncertainties associated with the data set used to derive the biphenyl RfD are more fully described below. The available database was determined to be inadequate for deriving a chronic inhalation RfC for biphenyl (see Section 5.2).

Selection of the critical effect for RfD determination. The critical effect selected for derivation of the RfD was skeletal anomalies in fetuses from rat dams administered biphenyl by gavage during GDs 6–15. An increased incidence of these anomalies was reported at doses ≥500 mg/kg-day; frank maternal toxicity, including death, was observed at the highest dose level (1,000 mg/kg-day). There is some degree of uncertainty regarding the toxicological significance of the reported skeletal anomalies (wavy or extra ribs and delayed ossification most commonly observed) and the influence of gavage dosing in the developmental toxicity study on human exposures. Supporting developmental toxicity studies are not available.

Dose-response modeling. BMD modeling was used to estimate the POD for the biphenyl RfD. BMD modeling has advantages over a POD based on a NOAEL or LOAEL because, in part, the latter are a reflection of the particular exposure concentration or dose at which a study was conducted. A NOAEL or LOAEL lacks characterization of the entire dose-response curve, and for this reason, is less informative than a POD obtained from BMD modeling. The selected model (i.e., the log-logistic model) provided the best mathematical fit to the experimental data set (as determined by the lowest AIC), but does not necessarily have greater biological support over the various other models included in BMDS. Other models in BMDS yielded estimates of the POD higher than the POD derived using the log-logistic model (by up to 5.8-fold).

Inadequate data to support RfC derivation. The available data do not support RfC derivation (see Section 5.2.1). Nevertheless, limited findings from human reports and from inhalation toxicity studies in experimental animals suggest that exposure to sufficiently high concentrations of biphenyl can potentially target the lungs, liver, and kidney. The lack of adequate data to derive an RfC represents a significant uncertainty for the evaluation of risks from exposure to inhaled biphenyl.

5.4. CANCER ASSESSMENT

As noted in Section 4.7.1, EPA concluded that there is "suggestive evidence of carcinogenic potential" for biphenyl. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) 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 this case, the carcinogenicity of biphenyl has been evaluated in two well-conducted 2-year bioassays in rats and mice (Umeda et al., 2005, 2002) that provide evidence of increased incidences of liver tumors in female BDF_1 mice and urinary bladder tumors in male F344 rats. Considering these data and uncertainty associated with the suggestive nature of the tumorigenic response, EPA 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 biphenyl is deemed appropriate.

5.4.1. Choice of Study/Data—with Rationale and Justification

No information was located regarding possible associations between oral exposure to biphenyl and cancer in humans. Two animal bioassays found statistically significant associations between lifetime oral exposure to biphenyl and tumor development. Biphenyl was associated with urinary bladder tumors in male, but not female, F344 rats (Umeda et al., 2002) and liver tumors in female, but not male, BDF_1 mice (Umeda et al., 2005). Tumor data for these two sites were selected for dose-response analysis.

No studies were identified that examined the association between inhalation exposure to biphenyl and cancer in humans or animals.

5.4.2. Dose-Response Data

The dose-response data for urinary bladder tumor formation resulting from lifetime oral exposure of male and female F344 rats (Umeda et al., 2002) are shown in Table 5-5. The dose-response data for liver tumor formation resulting from lifetime oral exposure of male and female BDF_1 mice (Umeda et al., 2005) are shown in Table 5-6. The datasets selected for dose-response analysis include urinary bladder transitional cell papilloma or carcinoma (combined) in the male F344 rats and liver adenoma or carcinoma (combined) in the female BDF_1 mice.

Table 5-5. Incidence data for tumors in the urinary bladder of male and female F344 rats exposed to biphenyl in the diet for 2 years

	Males				Females			
Biphenyl dietary concentration (ppm)	**0**	**500**	**1,500**	**4,500**	**0**	**500**	**1,500**	**4,500**
Calculated dose (mg/kg-d)[a]	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Tumor incidence[b]								
Transitional cell								
Papilloma	0/50	0/50	0/50	10/49[c]	0/50	0/50	0/50	0/50
Carcinoma	0/50	0/50	0/50	24/49[c]	0/50	0/50	0/50	0/50
Papilloma or carcinoma	050	0/50	0/50	31/49[c]	0/50	0/50	0/50	0/50

[a]Calculated doses based on TWA body weights (calculated from body weight data presented graphically in Figure 1 of (Umeda et al., 2002) and chronic reference food consumption values for F344 rats listed in Table 1-6 of U.S. EPA (1988).
[b]One high-dose male rat was excluded from the denominator because it died prior to week 52. It is assumed that this rat did not have a tumor and was not exposed for a sufficient time to be at risk for developing a tumor. Umeda et al. (2002) did not specify the time of appearance of the first tumor.
[c]Significantly different from control group ($p < 0.01$) according to Fisher's exact test.

Source: Umeda et al. (2002).

Table 5-6. Incidence data for liver tumors in male and female BDF_1 mice fed diets containing biphenyl for 2 years

	Dietary concentration of biphenyl (ppm)							
	Males				Females			
Biphenyl dietary concentration (ppm)	**0**	**667**	**2,000**	**6,000**	**0**	**667**	**2,000**	**6,000**
Reported dose (mg/kg-d)	**0**	**97**	**291**	**1,050**	**0**	**134**	**414**	**1,420**
Tumor incidence[a]								
Adenoma	8/50	6/49	7/49	3/50	2/48	3/50	12/49[b]	10/48[b]
Carcinoma	8/50	8/49	5/49	4/50	1/48	5/50	7/49[b]	5/48
Adenoma or carcinoma	16/50	12/49	9/49	7/50	3/48	8/50	16/49[c]	14/48[b]

[a]One low-dose, one mid-dose male, two control, one mid-dose, and two high-dose female mice were excluded from the denominators because they died prior to week 52. It is assumed that they did not have tumors and were not exposed for a sufficient time to be at risk for developing a tumor. Umeda et al. (2005) did not specify the time of appearance of the first tumor.
[b]Significantly different from controls ($p < 0.05$) according to Fisher's exact test as reported by Umeda et al. (2005).
[c]Significantly different from controls ($p < 0.01$) according to Fisher's exact test as reported by Umeda et al. (2005).

Source: Umeda et al. (2005).

5.4.3. Dose Adjustments and Extrapolation Method(s)

5.4.3.1. *Bladder Tumors in Male Rats*

There is strong evidence that the occurrence of urinary bladder tumors in male rats chronically exposed to biphenyl in the diet is a high-dose phenomenon involving occurrence of calculi in the urinary bladder leading to transitional cell damage, sustained regenerative cell proliferation, and eventual promotion of spontaneously initiated tumor cells in the urinary bladder epithelium (see Section 4.7.3.1 for a detailed discussion of the hypothetized mode of action for urinary bladder tumors in biphenyl-exposed male rats). No increased risk of bladder tumors is expected as long as the exposure to biphenyl is below the dose needed to form calculi (Cohen and Ellwein, 1992). As noted in the EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a nonlinear approach to dose-response analysis is used when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses. Therefore, consistent with the Cancer Guidelines, a nonlinear extrapolation approach for biphenyl-induced urinary bladder tumors was selected.

Based on the proposed mode of action, the available evidence indicates that doses below the oral RfD would not result in the sequence of events that includes calculus formation, consequent epithelial cell damage, and sustained regenerative cellular proliferation. Accordingly, the RfD of 0.2 mg/kg-day derived for noncancer effects of biphenyl was judged to be protective against an increased risk of biphenyl-induced urinary bladder cancer.

5.4.3.2. *Liver Tumors in Female Mice*

In the study report of their 2-year bioassay in BDF_1 mice, Umeda et al. (2005) provided average food consumption and biphenyl dose estimates for each exposure group (Table 1 of (Umeda et al., 2005). The study report did not include average body weights for the exposure groups. Therefore, the biphenyl concentration in the food was multiplied by the corresponding average daily food consumption value to determine the average daily biphenyl intake. Dividing this average daily biphenyl intake by the author-calculated daily dose yielded the average body weight that would have been used by the study authors to calculate the average daily biphenyl dose. Scaling factors were calculated using U.S. EPA (1988) reference body weight for humans (70 kg) and the average body weight for each dose group of female mice: (average body weight/70)$^{0.25}$ = scaling factor. The human equivalent dose (HED) was calculated as: HED = scaling factor × reported dose (Table 5-7).

Table 5-7. Scaling factors for determining HEDs to use for BMD modeling of female BDF_1 mouse liver tumor incidence data from Umeda et al. (2005)

Biphenyl dietary concentration (mg/kg food)	667	2,000	6,000
Reported dose (mg/kg-d)	134	414	1,420
Reported average food consumption (kg/d)	0.0058	0.0059	0.0059
Average mouse body weight (kg)[a]	0.0289	0.0285	0.0249
Scaling factor[b]	0.143	0.142	0.137
HED (mg/kg-d)[c]	19	59	195

[a](Biphenyl concentration in food [mg/kg food] × reported average food consumption [kg/day]) ÷ reported average daily dose of biphenyl (mg/kg-day) = calculated average mouse body weight (kg).
[b]Calculated using reference body weight for humans (70 kg) (U.S. EPA, 1988), and the average body weights for each dose group: mouse-to-human scaling factor = (average mouse body weight/70)$^{0.25}$.
[c]HED = reported dose × scaling factor.

The EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that when the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach. Accordingly, a linear approach to low-dose extrapolation for biphenyl-induced liver tumors in female mice was selected because the mode of action for this tumor site has not been established (see Section 4.7.3.2).

Incidence data for liver adenoma or carcinoma (combined) in the female mouse used to derive the oral slope factor are presented in Table 5-8. Tumor incidence data were adjusted to account for mortalities before 52 weeks; it was assumed that animals dying before 52 weeks were not exposed for sufficient time to be at risk for developing tumors (see footnote a in Table 5-8).

Table 5-8. Incidence of liver adenomas or carcinomas (combined) in female BDF_1 mice fed diets containing biphenyl for 2 years

Biphenyl dietary concentration (ppm)	0	667	2,000	6,000
HED (mg/kg-d)	**0**	**19**	**59**	**195**
Tumor incidence				
Adenoma or carcinoma (combined)	3/48[a]	8/50	16/49[a,b]	14/48[a,c]

[a]Two control, one mid-dose, and two high-dose female mice were excluded from the denominators because they died prior to week 52. It is assumed that they did not have tumors and were not exposed for a sufficient time to be at risk for developing a tumor. Umeda et al. (2005) did not specify the time of appearance of the first tumor.
[b]Significantly different from controls ($p < 0.05$) according to Fisher's exact test.
[c]Significantly different from controls ($p < 0.01$) according to Fisher's exact test.

Source: Umeda et al. (2005).

The multistage-cancer model in the EPA BMDS (version 2.1.2), using the extra risk option, was fit to the female mouse liver tumor incidence data. The multistage model has been used by EPA in the vast majority of quantitative cancer assessments because it is thought to reflect the multistage carcinogenic process and it fits a broad array of dose-response patterns. The multistage-cancer model was run for all polynomial degrees up to n-1 (where n is the number of dose groups including control). An extra risk of 10% tumor incidence was selected as the BMR. A 10% response is generally at or near the limit of sensitivity in most cancer bioassays, and in the case of biphenyl, corresponded to a POD near the lower end of the observed range in the Umeda et al. (2005) bioassay data. Adequate model fit was judged by three criteria: chi-square goodness-of-fit *p*-value ($p \geq 0.05$), visual inspection of the fit of the dose-response curve to the data points, and a value of <2 for the largest scaled residual for any data point in the dataset (including the control). If an adequate fit to the data was not achieved using the protocol above, then the other dichotomous models were fit to the data. If none of the models achieved an adequate fit for the full dataset, then the highest dose was dropped and the entire modeling procedure was repeated.

When liver tumor incidence data for all dose groups were modeled, none of the models in BMDS, including the multistage model, provided an adequate fit of the data (see Appendix D, Table D-2). The animals in the highest dose group, while exhibiting a statistically significantly increased incidence in liver tumors compared with controls, did not show a monotonic increase in tumor response compared with the responses at the lower doses. To better estimate responses in the low-dose region, the high-dose group was excluded as a means of improving the fit of the model in the region of interest. When the high-dose group was dropped, the multistage model provided an adequate fit to the data (see Appendix D, Table D-2). The BMD_{HED10} and $BMDL_{HED10}$ using this latter dataset were 18.7 and 12.2 mg/kg-day, respectively. See Appendix D for more information.

5.4.4. Oral Slope Factor and Inhalation Unit Risk

A low-dose linear extrapolation approach results in calculation of an oral slope factor that describes the cancer risk per unit dose of the chemical at low doses. The oral slope factor was calculated by dividing the risk (i.e., BMR of 10% extra risk) at the POD by the corresponding BMDL ($0.1/BMDL_{HED10}$). Using linear extrapolation from the $BMDL_{HED10}$, the human equivalent oral slope factor of 8.2×10^{-3} $(mg/kg\text{-}d)^{-1}$ (rounded to one significant figure, 8×10^{-3} $(mg/kg\text{-}d)^{-1}$) was derived for liver tumors in female BDF_1 mice (Table 5-9).

Table 5-9. POD and oral slope factor derived from liver tumor incidence data from BDF_1 female mice exposed to biphenyl in the diet for 2 years

Species/tissue site	BMD_{HED10} (mg/kg-d)	$BMDL_{HED10}$ (mg/kg-d)	Slope factor[a] (risk per [mg/kg-d])
Female mouse liver tumors	18.7	12.2	8.2×10^{-3}

[a]Human equivalent slope factor = $0.1/BMDL_{10HED}$; see Appendix C for details of modeling results.

This slope factor should not be used with exposures >12.2 mg/kg-day (the POD for this dataset), because above the POD, the fitted dose-response model better characterizes what is known about the carcinogenicity of biphenyl (i.e., the slope factor may not approximate the observed dose-response relationship adequately at exposure exceeding 12.2 mg/kg-day).

An inhalation unit risk for biphenyl was not derived in this assessment. The potential carcinogenicity of inhaled biphenyl has not been evaluated in human or animal studies, and route-to-route extrapolation was not possible in the absence of a PBPK model.

5.4.5. Uncertainties in Cancer Risk Values

5.4.5.1. *Oral Slope Factor*

A number of uncertainties underlie the cancer unit risk for biphenyl. Table 5-10 summarizes the impact on the assessment of issues such as the use of models and extrapolation approaches (particularly those underlying the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the effect of reasonable alternatives, the decision concerning the preferred approach, and its justification.

Table 5-10. Summary of uncertainties in the biphenyl cancer slope factor

Consideration/ approach	Impact on slope factor	Decision	Justification
Selection of data set	No other studies or data sets could be used to derive a slope factors	Umeda et al. (2005) studies were selected.	The bioassay by Umeda et al. (2005) was a well conducted experiment with sufficient dose groups (four dose groups, including control) and animal numbers (50 animals/sex) per group.
Cross-species scaling	Alternatives (i.e. scaling by [body weight] or [body weight]$^{2/3}$) could ↑ or ↓ slope factor	Administered dose was scaled to humans on the basis of equivalence of mg/kg$^{3/4}$-day (default approach)	There are no data to support alternatives. Use of [body weight]$^{3/4}$ for cross-species scaling is consistent with data that allow comparison of potencies in humans and animals, and it is supported by analysis of the allometric variation of key physiological parameters across mammalian species. No PBPK model is available to derive internal doses.
Extrapolation procedure for rat urinary bladder tumors	No impact on the slope factor because the MOA for male rat bladder tumors does not support low-dose linear extrapolation.	Nonlinear extrapolation. The RfD of 0.2 mg/kg-day is considered to protect against the risk of urinary bladder tumors.	Available MOA data for urinary bladder tumors support nonlinearity (i.e., that bladder tumor is a high-dose phenomena, and is closely related to calculi formation in the urinary bladder of male rats).
Extrapolation procedure for mouse liver tumors	Departure from EPA's *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ slope factor by an unknown extent	Multistage model to determine the POD, linear low-dose extrapolation from POD (default approach)	Available MOA data do not inform selection of dose-response model; linear approach in absence of clear support for an alternative is generally consistent with scientific deliberations supporting EPA's *Guidelines for Carcinogen Risk Assessment.*
Human relevance of female mouse liver tumor data	Human risk could ↑or ↓, depending on relative sensitivity	Liver tumors in female mice are relevant to human exposure	It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown.
Model uncertainty	For poorly fitting liver tumors dataset, alternatives could ↓ or ↑ slope factor	Drop highest dose of the liver tumors dataset.	Model options explored with full liver tumor datasets did not generate a $p \geq 0.05$, which is one of the indications of dropping the highest dose according to the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000b).
Statistical uncertainty at POD	↓ slope factor 1.5-fold if BMD_{10} used rather than $BMDL_{10}$	BMDL (default approach for calculating plausible upper bound)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on dose.
Human population variability / sensitive subpopulations	Low-dose risk ↑ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity in metabolism or response, including whether children are more sensitive.

$BMDL_{10}$ = 95% lower confidence limits on the doses associated with a 10% extra risk of cancer incidence.

The uncertainties presented in Table 5-10 have a varied impact on risk estimates. Some suggest risks could be higher than was estimated, while others would decrease risk estimates or have an impact of an uncertain direction. Several uncertainties are quantitatively characterized for the significantly increased rodent tumors. These include the statistical uncertainty in the multistage modeling estimate. Due to limitations in the data, particularly regarding the MOA and relative human sensitivity and variability, the quantitative impact of other uncertainties of potentially equal or greater impact has not been explored. As a result, an integrated quantitative analysis that considers all of these factors was not undertaken.

5.4.5.2. *Inhalation Unit Risk*

The potential carcinogenicity of inhaled biphenyl has not been assessed. Therefore, a quantitative cancer assessment for biphenyl by the inhalation pathway was not performed.

5.4.6. Previous Cancer Assessment

In the previous IRIS cancer assessment (U.S. EPA, 1991), biphenyl was listed in Group D; not classifiable as to human carcinogenicity based on no human data and inadequate studies in mice and rats. Neither an oral slope factor nor inhalation unit risk was derived in the previous cancer assessment.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

6.1.1. Noncancer

Toxicokinetic studies of animals indicate that orally administered biphenyl is rapidly and readily absorbed, distributed widely to tissues following absorption, and rapidly eliminated from the body, principally as conjugated hydroxylated metabolites in the urine (Meyer, 1977; Meyer and Scheline, 1976; Meyer et al., 1976a; Meyer et al., 1976b). Data for absorption, distribution, and elimination are not available for inhaled or dermally applied biphenyl. Metabolism to a range of hydroxylated metabolites has been demonstrated in in vitro systems with rat and human cells and tissues. Human metabolism of biphenyl appears to be qualitatively similar to metabolism in the rat, although some reports of quantitative differences are available (Powis et al., 1989; Powis et al., 1988; Benford et al., 1981).

Available human health hazard data consist of limited assessments of workers exposed to biphenyl during the production or use of biphenyl-impregnated fruit wrapping paper in which signs of hepatic and nervous system toxicity were observed.

Chronic oral studies in rats and mice identify the liver and urinary system as principal targets of biphenyl toxicity, the rat kidney being the most sensitive. In chronically exposed rats, nonneoplastic kidney lesions (simple transitional cell hyperplasia in the renal pelvis and hemosiderin deposits) were found in females at ≥1,500 ppm biphenyl in the diet (128 mg/kg-day), and urinary bladder tumors, associated with urinary bladder calculi and transitional cell hyperplasia, were found in males, but not females, at the highest tested concentration, 4,500 ppm (378 mg/kg-day) (Umeda et al., 2002). Several other rat studies provide supporting evidence that the kidney and other urinary tract regions are sensitive targets for biphenyl in rats (Shiraiwa et al., 1989; Ambrose et al., 1960; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953). In chronically exposed BDF_1 mice, increased incidence of nonneoplastic effects on the kidney (mineralization) and liver (increased activities of plasma ALT and AST) were found in females exposed to ≥2,000 ppm biphenyl in the diet (414 mg/kg-day) (Umeda et al., 2005). In contrast, no exposure-related nonneoplastic or neoplastic effects on the liver or kidney were found in female ddY mice exposed to 5,000 ppm biphenyl in the diet for 2 years (Imai et al., 1983) or in $B6C3F_1$ and $B6AKF_1$ mice exposed to 517 ppm biphenyl in the diet for 18 months (Innes et al., 1969; NCI, 1968). In the only available developmental toxicity study for biphenyl, increased incidences of litters with fetuses showing skeletal anomalies were reported following exposure of pregnant rats to gavage doses ≥500 mg/kg-day on GDs 6–15 (Khera et al., 1979).

Biphenyl effects on reproductive function in rats have been reported at a higher exposure level than the lowest exposure levels associated with urinary tract, liver, or developmental

toxicity. No exposure-related effect on the number of dams with litters was found following exposure of male and female albino rats to up to 5,000 ppm biphenyl in the diet (525 mg/kg-day) for 11 or 60 days prior to mating (Ambrose et al., 1960). In a three-generation rat study, decreased fertility, decreased number of pups/litter, and decreased pup body weight were observed at 10,000 ppm biphenyl in the diet (947 mg/kg-day), but not at ≤1,000 ppm (Dow Chemical Co, 1953).

No chronic inhalation toxicity studies in animals are available. In subchronic inhalation toxicity studies, respiratory tract irritation and increased mortality following exposure to dusts of biphenyl (7 hours/day, 5 days/week for up to about 90 days) were reported in mice exposed to 5 mg/m^3 and in rats exposed to 300 mg/m^3, but not in rabbits exposed to 300 mg/m^3 (Deichmann et al., 1947; Monsanto, 1946). Congestion or edema of the lung, kidney, and liver, accompanied by hyperplasia with inflammation of the trachea, was reported in CD-1 mice exposed to biphenyl vapors at 25 or 50 ppm (158 or 315 mg/m^3) for 13 weeks (Sun, 1977a).

6.1.2. Cancer

No assessments are available regarding possible associations between exposure to biphenyl and increased risk of cancer in humans.

In a 2-year study of F344 rats administered biphenyl in the diet, significantly increased incidences of urinary bladder tumors in males were observed at the highest dose level (378 mg/kg-day). There is strong evidence that the occurrence of urinary bladder tumors in the male rats is a high-dose phenomenon involving occurrence of calculi in the urinary bladder leading to transitional cell damage, sustained regenerative cell proliferation, and eventual promotion of spontaneously initiated tumor cells in the urinary bladder epithelium. Urinary bladder calculi in the high-dose (438 mg/kg-day) female rats were observed at much lower incidence and were different in physical appearance and chemical composition; furthermore, there were no urinary bladder tumors in any of the biphenyl-exposed female rats.

In a 2-year study of BDF_1 mice administered biphenyl in the diet, the incidence of liver tumors in female mice was significantly increased at doses ≥414 mg/kg-day, but not in males at doses up to and including 1,050 mg/kg-day. Available data are insufficient to establish a mode of action for liver tumors in female mice.

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for biphenyl provides "suggestive evidence of carcinogenic potential" at environmentally relevant exposure levels in humans where the formation of urinary bladder tumors would not be expected to occur. This cancer descriptor is based on an increase in the incidence of liver tumors (hepatocellular adenomas and carcinomas) in female BDF_1 mice (Umeda et al., 2005) and urinary bladder tumors (transitional cell papillomas and carcinomas) in male F344 rats (Umeda et al., 2002) exposed to biphenyl in the diet for 104 weeks, as well as information on mode of carcinogenic action.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

The RfD of 0.2 mg/kg-day was based on an increased incidence of litters with fetal skeletal anomalies from Wistar rat dams administered biphenyl by gavage on GDs 6–15 (Khera et al., 1979). The $BMDL_{10}$ of 20 mg/kg-day was selected as the POD. To derive the RfD, the POD was divided by a total UF of 100 (10 for animal-to-human extrapolation and 10 for human interindividual variability in susceptibility). The interspecies uncertainty factor was applied to account for the lack of quantitative information to assess toxicokinetic and toxicodynamic differences between animals and humans. The intraspecies UF was applied to account for the lack of information regarding the range of responses to biphenyl in the human population.

The overall confidence in the RfD assessment is medium to high. Confidence in the principal study (Khera et al., 1979) is medium to high. The design, conduct, and reporting of this developmental toxicity study in Wistar rats were adequate; however, only litter average data were available that did not permit a nested analysis based on individual fetal data. Confidence in the database is high. The database is robust in that it includes chronic-duration oral exposure studies in several rat and mouse strains, a developmental toxicity study in Wistar rats, and one- and three-generation reproductive toxicity studies in rats.

6.2.2. Noncancer/Inhalation

No inhalation RfC was derived due to the lack of studies of biphenyl toxicity following chronic exposure and studies involving subchronic exposure that were inadequate for RfC derivation. Repeated exposure of mice to biphenyl vapors for 13 weeks resulted in high incidences of pneumonia and tracheal hyperplasia, and high incidences of congestion and edema in the lungs, liver, and kidney (Sun, 1977a); however, study limitations and lack of supporting data preclude the use of this study for deriving an RfC for biphenyl. Study limitations include highly variable biphenyl exposure concentrations during the first half of the study, high mortality after 46 exposures in one group of biphenyl-exposed mice due to an overheating event and cannibalization that necessitated the use of replacement animals, and limitations in the reporting of histopathological findings.

6.2.3. Cancer/Oral

The oral slope factor of 8×10^{-3} per mg/kg-day is based on the tumor response in the liver of female BDF_1 mice exposed to biphenyl in the diet for 2 years (Umeda et al., 2005). The slope factor was derived by linear extrapolation from a human equivalent $BMDL_{10}$ of 12.2 mg/kg-day for liver adenomas or carcinomas.

A nonlinear extrapolation approach for biphenyl-induced urinary bladder tumors in male rats was used because the available mode of action information indicates that the induction of urinary bladder tumors is a high-dose phenomenon involving occurrence of calculi in the urinary bladder leading to transitional cell damage, sustained regenerative cell proliferation, and eventual promotion of spontaneously initiated tumor cells in the urinary bladder epithelium. As long as the dose is below that which is needed to form calculi, no increased risk of bladder tumors is expected. Therefore, the RfD of 0.2 mg/kg-day derived for noncancer effects of biphenyl was judged to be protective against increased risk of biphenyl-induced urinary bladder cancer.

6.2.4. Cancer/Inhalation

No human or animal data on the potential carcinogenicity of inhaled biphenyl are available. Therefore, a quantitative cancer assessment for biphenyl by the inhalation pathway was not performed.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

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APPENDIX B. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

B.1. EFFECTS ON THE URINARY TRACT OF RATS

Urinary tract effects in male rats chronically exposed to biphenyl in the diet are associated with the formation of urinary bladder calculi. Mechanistic studies performed by Ohnishi and coworkers (2001; 2000a; 2000b) were designed to identify urinary metabolites of biphenyl, to assess conditions leading to calculi formation, and to determine the composition of urinary crystals and calculi. Ohnishi et al. (2000a) identified sulphate conjugates of mono- and dihydroxy biphenyl metabolites in urine and urinary crystals from F344 rats treated with biphenyl and $KHCO_3$ (to elevate the pH and K^+ concentration of the urine). Male F344 rats (five per group) were administered a diet containing 1.6% biphenyl and 5% potassium bicarbonate for 7 days (Ohnishi et al., 2000a). Urine was collected on days 6 and 7 and pooled. Urinary crystals (i.e., precipitates) were collected, dissolved in acetonitrile, and analyzed by HPLC to identify metabolites or by inductively coupled plasma spectroscopy to identify inorganic elements. As shown in Table B-1, biphenyl sulphate conjugates in the urine consisted primarily of 3,4-dihydroxybiphenyl-3-O-sulphate (40.9% of the total biphenyl sulphate conjugates) and 3-hydroxybiphenyl (23.4%). No bisulphates were observed (Ohnishi et al., 2000a). In contrast, about 90% of sulphate conjugates in urinary crystals were 4-hydroxybiphenyl-O-sulphate, and only 3.9 and 1.06% were 3,4-dihydroxybiphenyl-3-O-sulphate and 3-hydroxybiphenyl, respectively. In a follow-up study, Ohnishi et al. (2000b) evaluated the composition of urinary calculi in male and female rats exposed to 4,500 ppm biphenyl in the diet for 104 weeks. Urinary calculi in chronically exposed male rats were composed mainly of 4-hydroxybiphenyl-O-sulphate, whereas calculi in female rats were composed primarily of 4-hydroxybiphenyl and potassium sulphate, the hydrolysis products of 4-hydroxybiphenyl-O-sulphate (Ohnishi et al., 2000b).

Table B-1. Content of biphenyl sulphate conjugates in urine and urinary crystals from F344 rats treated with biphenyl and potassium bicarbonate (to elevate the pH and K^+ concentration of the urine)

Biphenyl sulphate conjugates	Urine (%)	Urine crystals (%)
2-Hydroxybiphenyl-O-sulphate	3.32[a]	0.06
3-Hydroxybiphenyl-O-sulphate	23.37	1.06
4-Hydroxybiphenyl-O-sulphate	11.94	89.45
4,4'-Dihydroxybiphenyl-O-sulphate	7.17	3.11
2,5-Dihydroxybiphenyl-O-sulphate	5.62	0.02
3,4-Dihydroxybiphenyl-3-O-sulphate	40.88	3.90
3,4- Dihydroxybiphenyl-4-O-sulphate	2.27	2.28
2,3- Dihydroxybiphenyl-3-O-sulphate	5.43	0.12

[a]The component fraction (%) for each of the sulphate conjugates was estimated from the ratio of the liquid chromatography tandem MS peak area of the sulfate to the total area.

Source: Ohnishi et al. (2000a).

Using the same experimental protocol as that described in Ohnishi et al. (2000a), but adding potassium bicarbonate (5%), potassium chloride (5%), or sodium bicarbonate (8%) to the diet for 13 weeks, Ohnishi et al. (2001) reported hydronephrosis and blood in the urine only in those animals receiving biphenyl plus potassium bicarbonate. Feed consumption was not affected by the dietary additions, while water intake was greatly increased in all groups of animals that received biphenyl and/or salts. Neither high urinary potassium levels alone, as induced by co-feeding of potassium chloride, nor high urinary pH alone, as induced by co-feeding of sodium bicarbonate, were sufficient to cause kidney damage. It was concluded that a combination of high urinary pH and high potassium levels was necessary to cause precipitation of biphenyl sulphate. It was proposed that the crystalline precipitate caused obstruction that led to hydronephrosis or damaged the transitional epithelium in the bladder causing hyperplasia.

B.2. EFFECTS ON THE LIVER OF MICE

Based on findings of biphenyl-induced liver tumors in female BDF_1 mice administered high dietary concentrations of biphenyl for 2 years (Umeda et al., 2005) (see Section 4.2.1.2.2), a 13-week oral study was performed to assess whether peroxisome proliferation might be induced (Umeda et al., 2004b). Groups of male and female BDF_1 mice (10/sex/group) were administered biphenyl in the diet at six different concentrations ranging from 500 to 16,000 ppm. Biphenyl concentrations ≥8,000 ppm resulted in significantly decreased final body weights of males and females. Significantly increased liver weights were noted in the 8,000 and 16,000 ppm groups of female mice. Evidence of peroxisome proliferation was restricted to the 16,000 ppm group of female mice and included light microscopy findings of clearly enlarged hepatocytes filled with eosinophilic fine granules and electron microscopy confirmation that the granules corresponded

to increased numbers of peroxisomes. Light microscopy of livers from rats exposed to concentrations ≤8,000 ppm showed no indications of proliferation of peroxisomes. There were no indications of other biphenyl-induced liver effects in any of the groups of male mice.

B.3. ESTROGENIC EFFECTS

Several biphenyl derivatives display estrogenic activity. Schultz et al. (2002) used the *Saccharomyces cerevisiae/LacZ* reporter assay to study the estrogenic activity of 120 chemicals to identify chemical structures that impart estrogenic activity to a molecule. Chemicals without a hydroxy group, among them biphenyl, were inactive in this assay. The estrogenic activities of biphenyl metabolites in this assay were 4,4′-dihydroxybiphenyl (median effective concentration = (2.6×10^{-7} M) > 4-hydroxybiphenyl (1.2×10^{-6} M) > 3-hydroxybiphenyl (9.2×10^{-6} M) > 2-hydroxybiphenyl (1.8×10^{-5} M). Estrogenic activities of the corresponding hydroxylated di-, tri-, or tetrachlorobiphenyl metabolites were approximately two orders of magnitude higher, provided there were no chlorines and hydroxy groups on the same ring.

Kitamura et al. (2003) used MCF-7 cells transfected with an estrogen receptor-luciferase reporter construct to test biphenyl and its metabolites for estrogenic activity. The starting point for this investigation was the structural similarity between hydroxylated metabolites of biphenyl and of 2,2-diphenyl propane, the 4,4′-dihydroxy metabolite of which is bisphenol A, a known endocrine disrupter. Biphenyl per se displayed no estrogenic activity in this assay. Metabolites of biphenyl formed by liver microsome preparations were identified after solvent extraction from reaction media by HPLC-MS. The compounds were also tested in an in vitro competitive estrogen receptor binding assay. The biphenyl metabolites, 2-, 3-, 4-hydroxybiphenyl, and 4,4′-dihydroxybiphenyl, all exhibited estrogenic activity when the cell culture contained microsomes from 3-methylcholanthrene-induced rat livers and to a lesser extent, phenobarbital-induced rat livers, in the presence of NADPH. In the competitive estrogen receptor binding assay, 4,4′-dihydroxybiphenyl displayed weak binding affinity, while biphenyl and its monohydroxy metabolites did not show any activity. 4,4′-Dihydroxybiphenyl is one of two major biphenyl metabolites in rats and mice (Halpaap-Wood et al., 1981a, b; Meyer and Scheline, 1976), suggesting that high doses of biphenyl, in the form of this metabolite, might induce some minor estrogenic effect.

B.4. EFFECTS ON APOPTOSIS

Kokel and Xue (2006) tested a series of benzenoid chemicals (including mesitylene, cyclohexane, benzene, toluene, and biphenyl) for their ability to suppress apoptosis in the nematode, *Caenorhabditis elegans*, a model suitable for the characterization of carcinogens that act by way of apoptosis inhibition. The study included wild type and three strains of *C. elegans* mutants; the ced-3(n2438) mutant (which carries a partial loss-of-function mutation in the ced-

3 gene), the ced-3(n2273) mutant (also partly defective in cell death), and the ced-(n2433) mutant (a strong loss-of-function ced-3 mutant). Effects on apoptosis were assessed by counting the numbers of cells that should have died during embryogenesis, but inappropriately survived. The results indicated that these chemicals did not significantly affect apoptosis in wild type *C. elegans*. However, inhibition of apoptosis was apparent in mutant strains ced-3(n2438) and ced-3(n2273) exposed to benzene, toluene, or biphenyl. The study authors interpreted these results as indicative of apoptosis-inhibitory activity that does not depend on mutations in a specific cell-death gene. A lack of apparent apoptosis-inhibitory activity in the strong loss-of-function ced-3(n2433) mutant was interpreted as indicative that inhibition of apoptosis, rather than transformation of cell fates, caused the increase in extra cell observed in the other two mutant strains. All three chemicals also displayed embryotoxicity. Biphenyl and naphthalene were both shown to suppress apoptosis in *C. elegans* mutant strain ced-3(n2438) by causing overexpression of the CED-3 caspase. The authors proposed that benzenoid chemicals that can form quinones suppress apoptosis in *C. elegans* via this reactive intermediate, although this was proven only for benzene, toluene, and naphthalene.

Regulation of apoptosis during embryogenesis is critical, and a recent study by Tan et al. (2011) showed that inhibition of apoptosis during this stage of development may have detrimental effects on the nervous system. No literature was identified, however, that specifically supports an association between inhibition of apoptosis by biphenyl and effects on embryogenesis.

B.5. MITOCHONDRIAL EFFECTS

Nishihara (1985) assessed the effects of biphenyl on the respiratory and energy linked activities of rat liver mitochondria that had been isolated from male Wistar rats. Biphenyl (5–60 µg/mL in acetone solvent) was added to liver mitochondria and effects on rates of succinate oxidation and α-ketoglutarate/malate oxidation were assessed by measuring oxygen consumption. Solvent controls were included in the study. Biphenyl significantly inhibited state 3 respiration at concentrations ≥20 µg/mL. The inhibition was greater for α-ketoglutarate/malate oxidation than for succinate oxidation. State 4 respiration was significantly stimulated by biphenyl; the effect was greater in magnitude for succinate than for α-ketoglutarate/malate oxidation. Biphenyl also altered mitochondrial membrane permeability, as evidenced by the instantaneous release of endogenous K^+, leading to instantaneous dissipation of the mitochondrial membrane potential. Inhibition of state 3 respiration is generally considered to reflect an interference with electron transport. The study author suggested that the biphenyl-induced stimulation of state 4 respiration may be explained by an uncoupling action on respiration.

B.6. GENOTOXICITY

Biphenyl. The results of genotoxicity studies of biphenyl are summarized in Table B-2. Reverse mutation assays using *Salmonella typhimurium* and *Escherichia coli* provide consistently negative results both with and without the addition of a mammalian metabolic activation system (rat S9 mix). Biphenyl was not genotoxic in a host-mediated deoxyribonucleic acid (DNA) repair assay of *E. coli* in the presence of S9 (Hellmér and Bolcsfoldi, 1992). In rec assays of *Bacillus subtilis*, two studies reported negative results both with and without S9 (Garrett et al., 1986; Kojima and Hiraga, 1978), one study reported negative results without S9 (Kawachi et al., 1980), and one study reported equivocal results with S9 (Hanada, 1977). Biphenyl was reported to induce mitotic recombination both with and without S9 in *Saccharomyces cerevisiae* strain D3 (1988), but not in *S. cerevisiae* strain Diploid D7 (Garrett et al., 1986).

Table B-2. Genotoxicity test results for biphenyl

Organism	Strain or test system	Endpoint	Test substance concentrations	Metabolic activation[a]		Reference
				+S9	–S9	
Bacterial and prokaryotic assays						
S. typhimurium	TA98, 100	Mutation	Not specified	–	NT	Bos et al. (1988)
	TA98, 100, 1535, 1538		Not specified	–	NT	Purchase et al. (1978)
	TA98, 100		Not specified	–	–	Kawachi et al. (1980)
	TA97, 98, 100		1–100 µg/plate	–	–	Brams et al. (1987)
	TA98, 1535		5–1,000 µg/plate[b]	–	NT	Narbonne et al. (1987)
	TA98, 100, YG1041		5–250 µg/plate[b]	–	–	Chung and Adris (2003, 2002)
	TA98, 100, 1535,1537, 1538, C3076, D3052, G46		0.1–1,000 µg/mL	–	–	Cline and McMahon (1977)
	TA98, 100, 1537		$1–10^5$ µg/mL[b]	–	–	Garrett et al. (1986); Waters et al. (1982)
	TA98, 100		25–800 µg/plate	–	–	Glatt et al. (1992)
	TA1535, 1536, 1537-1, 1538-1		Units provided in Japanese	–		Hanada (1977)
	TA98, 100		1–1,000 µg/plate	–	–	Kojima and Hiraga (1978)
	TA98, 100, 1535, 1537		1–100 µg/plate	–	–	Haworth et al. (1983)
	TA98, 100		0.15–2 µg/plate	–	–	Houk et al. (1989)
	TA98, 100, 1535, 1537, 2637		Up to 5 mg/plate	–	NT	Ishidate et al. (1984)
	TA98, 100, 1532, 1535, 1537, 1538, 2636		0.1–500 µg/plate[b]	–	–	Pagano et al. (1988; 1983)
	C3076, D3052, G46, TA98, 1000, 1535, 1537, 1538		10^4-fold range	–	–	Probst et al. (1981)
	TA98, 100, 1535, 1537, 1538, 1978		77 µg/plate	–	–	Westinghouse (1977)
E. coli	Chromotest	Mutation	2.4–154 µg/mL	–	–	Brams et al. (1987)
	WP2, WP2 uvrA$^-$		1–1,000 µg/mL	–	–	Cline and McMahon (1977)
	WP2, WP2 uvrA$^-$		10^4-fold range	–	–	Probst et al. (1981)
	WP uvrA$^-$, polA$^-$		$1–10^5$ µg/mL	–	–	Garrett et al. (1986)
	B/γ WP_2try^-, B/γ $WP_2try^-hcr^-$		Units provided in Japanese	–		Hanada (1977)
	B/γ $WP_2try^-hcr^-$		≤1,000 µg/mL[b]	–	–	Kojima and Hiraga (1978)
	K-12 uvrB/recA$^+$ K-12 uvrB/recA$^-$	Host-mediated DNA repair	Up to 161 mM	–	NT	Hellmér and Bolcsfoldi (1992)

Table B-2. Genotoxicity test results for biphenyl

Organism	Strain or test system	Endpoint	Test substance concentrations	Metabolic activation[a]		Reference
				+S9	–S9	
B. subtilis	Not given	Rec assay	Not specified	NT	–	Kawachi et al. (1980)
	recA$^-$		1–10^5 µg/mL	–	–	Garrett et al. (1986)
	H17 (rec$^+$) M45 (rec$^-$)		Units provided in Japanese	+/– +/–		Hanada (1977)
	H17 (rec$^+$) M45 (rec$^-$)		1 or 10 mg	–	–	Kojima and Hiraga (1978)
S. cerevisiae	D3	Mitotic recombination	1–10^5 µg/mL	–	–	Garrett et al. (1986)
	Diploid D7		10^{-5} or 10^{-3} M 10^{-5} M[a]	+	+	Pagano et al. (1988)
Tests with cultured mammalian cells						
Hamster	V79	Mutation	5–100 µg/mL 100 µg/mL[b]	+	–	Glatt et al. (1992)
	DON	SCEs	0.1–1 mM		–	Abe and Sasaki (1977)
	CHL		Not specified	NT	–	Kawachi et al. (1980)
		CAs	Not specified	NT	–	Kawachi et al. (1980)
			Up to 25 µg/mL	–	NT	Ishidate et al. (1984)
			Up to 60 µg/mL	–	NT	Ishidate and Odashima (1977)
			75–125 µg/mL	+	–	Sofuni et al. (1985)
	DON		0.1–1 mM		–	Abe and Sasaki (1977)
	Kidney	Cell transformation	0.025–250 µg/mL	–	NT	Purchase et al. (1978)
	V79		≤100 µg/mL	+	–	Glatt et al. (1992)
	CHO	CAs	3.1–200 µg/mL 100 µg/mL[a]	–		Yoshida et al. (1978)
Human	Peripheral blood lymphocytes	SCEs	10–70 µL/mL	NT	+/–	Rencüzoğullari et al. (2008)
		CAs	10–70 µL/mL	NT	+	
		Micronuclei	10–70 µL/mL	NT	+	
	Diploid lung fibroblast	Cell transformation	0.025–250 µg/mL	–	NT	Purchase et al. (1978)
	Liver-derived cells		0.025–250 µg/mL	–	NT	Purchase et al. (1978)
	HSBP diploid lung fibroblast	DNA repair	100 µM	–		Snyder and Matheson (1985)
	WI-38 lung fibroblasts	UDS	1–10^5 µg/mL	–	–	Garrett et al. (1986); Waters et al. (1982)
Rat	Primary hepatocyte	UDS	0.01–100 µM	–		Hsia et al. (1983a, b)
			100 µM	–		Probst et al. (1981)
		Excision repair	0.01–1,000 µM	–		Brouns et al. (1979)
		DNA repair	100 µM[c]	–		Williams et al. (1989)
	Immortalized liver epithelial cells	HGPRT mutation	100 µM	–		Williams (1980)

Table B-2. Genotoxicity test results for biphenyl

Organism	Strain or test system	Endpoint	Test substance concentrations	Metabolic activation[a]		Reference
				+S9	–S9	
Mouse	L5178Y/TK$^{+/-}$	Mutation	50–500 µM 150 µM[a]		–	Garberg et al. (1988)
			50–1,500 µM 500 µM[a]	+		
			98.7–395 µM 98.7 µM[a]		+[d]	Wangenheim and Bolcsfoldi (1988, 1986)
			5–60 µM 10 µM[a]	+[d]		
In vivo tests						
Rat	Bone marrow	SCEs	Not specified	–		Kawachi et al. (1980)
		CAs	Not specified	–		
Mouse	CD-1/stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	DNA damage, Comet assay	10–2,000 mg/kg	+		Sasaki et al. (2002)
Mouse	CD-1/stomach, liver, kidney, bladder, lung, brain, bone marrow	DNA damage, Comet assay	2,000 mg/kg	+		Sasaki et al. (1997)
Silkworm		Mutation	Not specified	–		Kawachi et al. (1980)

[a]Lowest concentration resulting in cytotoxicity.
[b]Lowest concentration resulting in precipitation.
[c]Highest concentration not causing cytotoxicity.
[d]Positive result only at cytotoxic concentrations.

CA = chromosomal aberration; CHL = Chinese hamster lung; CHO = Chinese hamster ovary; HGPRT = hypoxanthine guanine phosphoribosyl transferase; NT = not tested; +/– = weakly positive or equivocal result; empty cell = no information available; SCE = sister chromatid exchange; UDS = unscheduled DNA synthesis

Assays of biphenyl-exposed cultured mammalian cells provide mixed genotoxicity results. In the absence of exogenous metabolic activation, biphenyl produced negative results for sister chromatid exchanges (SCEs) and/or chromosomal aberrations (CAs) in the DON Chinese hamster cell line (Abe and Sasaki, 1977) or Chinese hamster lung (CHL) fibroblasts (Sofuni et al., 1985; Kawachi et al., 1980); unscheduled DNA synthesis (UDS) (Garrett et al., 1986; Hsia et al., 1983a, b) (Waters et al., 1982; Probst et al., 1981), excision repair (Brouns et al., 1979), and DNA repair in rat hepatocytes (Williams et al., 1989); and hypoxanthine guanine phosphoribosyl transferase (HGPRT) mutation in rat immortalized liver epithelial cells (Williams, 1980) In the presence of S9 mix, biphenyl produced negative results for CAs in CHL fibroblasts (Ishidate et al., 1984; Ishidate and Odashima, 1977) or Chinese hamster ovary (CHO) cells (Yoshida et al., 1978); DNA repair in human HSBP diploid lung fibroblasts (Snyder and Matheson, 1985); cell

transformations in Chinese hamster kidney cells (Purchase et al., 1978) and human diploid lung fibroblasts (Purchase et al., 1978); and UDS in human lung WI-38 lung fibroblasts (with or without S9) (Garrett et al., 1986).

Positive results were obtained for CAs in CHL fibroblasts (Sofuni et al., 1985) and mutations and transformations in Chinese hamster V79 cells (Glatt et al., 1992) in the presence, but not absence, of S9. Biphenyl induced forward mutations in mouse L5178Y/TK$^{+/-}$ lymphoma cells with and without S9 (Wangenheim and Bolcsfoldi, 1988, 1986); another study provided similar results in the presence, but not the absence, of S9 (Garberg et al., 1988). Significant increases in SCEs (less than twofold higher than solvent controls), CAs (two- to fourfold higher than solvent controls), and micronuclei (approximately 2.5-fold higher than solvent controls) were reported in human peripheral blood lymphocytes exposed to biphenyl for 24–48 hours at concentrations ≥50 µL/mL (Rencüzoğullari et al., 2008).

Evaluations of the potential genotoxicity of biphenyl in vivo have been performed in rats, mice, and silkworms. Biphenyl did not induce SCEs or CAs in bone marrow cells of rats or mutations in silkworms, but limited information is available for these studies (Kawachi et al., 1980). In a Comet assay, positive results were reported for DNA damage in stomach, blood, liver, bone marrow, kidney, bladder, lung, and brain cells of CD-1 mice administered single doses of 2,000 mg biphenyl/kg (Sasaki et al., 2002; Sasaki et al., 1997). It is unknown if the DNA damage was caused by direct reaction with biphenyl or its metabolites, or by indirect damage from cytotoxicity or ROS generated from redox cycling of hydroquinone metabolites.

Biphenyl metabolites. Table B-3 summarizes results from genotoxicity tests of several biphenyl metabolites, 2-hydroxybiphenyl (also known as *o*-phenylphenol), 4-hydroxybiphenyl (the principal metabolite of biphenyl), and 2,5-dihydroxybiphenyl. 2-Hydroxybiphenyl and its sodium salt have received the most research attention because they are used as fungicides and anti-bacterial agents and have been found to cause urinary bladder tumors in male F344 rats with chronic exposure to high concentrations in the diet (Balakrishnan et al., 2002; Kwok et al., 1999) for review).

Table B-3. Genotoxicity test results for biphenyl metabolites

Organism	Strain or test system	Endpoint	Test substance concentrations	Metabolic activation[a]		Reference
				+S9	–S9	
2-Hydroxybiphenyl in vitro tests						
S. typhimurium	TA98, TA100	Mutation	Not specified	–	–	Kawachi et al. (1980)
	TA98, 100, 1535, 1537		3.3–250 µg/plate	–	–	Haworth et al. (1983)
	TA98, 100		1–1,000 µg/plate	–	–	Kojima and Hiraga (1978)
	TA97a, 102		1–100 µg/plate	–	–	Fujita et al. (1985)
	TA98, 100, 1535, 1537, 2637		Up to 0.5 mg/plate	–	NT	Ishidate et al. (1984)
	TA98, 100		Not specified	+/–	+/–	Nishioka and Ogasawara (1978)
	TA1535, 1537-1, 1538-1 TA1536		Units provided in Japanese	+/– +		Hanada (1977)
E. coli	B/γ $WP_2try^-hcr^-$ B/γ WP_2try^-		1–1,000 µg/mL 1,000 µg/mL[a]	+/–	+/–	Kojima and Hiraga (1978)
	WP2 lacking catalase and superoxide dismutase	Streptomycin resistance mutation	0–10 µM	NT	+	Tani et al. (2007)
	WP2, WP2 $uvrA^-$, CM571, WP100	DNA repair	Not specified	+	+	Nishioka and Ogasawara (1978)
B. subtilis	Not given	Rec assay	10–10,000 mg/plate	–	–	Kawachi et al. (1980)
	H17 (rec^+) M45 (rec^-)		Units provided in Japanese	+	+	Kojima and Hiraga (1978); Hanada (1977)
Hamster	CHL	CAs	Not specified	NT	–	Kawachi et al. (1980)
			Up to 0.05 mg/mL	–	NT	Ishidate et al. (1984)
	CHO		3.1–200 µg/mL 94 µg/mL[a]	–		Yoshida et al. (1978)
Rat	Liver DNA	DNA adducts, [^{32}P]-post labeling method	1 mM, in presence of rat skin homogenate, CYP, or prostaglandin synthase activation systems	+[b]		Pathak and Roy (1993)
2-Hydroxybiphenyl in vivo tests						
Rat	Bone marrow	SCEs	Not specified	–		Kawachi et al. (1980)
	F344/bladder epithelium	Micronuclei Hyperdiploidy/ hypodiploidy Cell proliferation	2,000 ppm in diet, 14 days	+ – +		Balakrishnan et al. (2002)

Table B-3. Genotoxicity test results for biphenyl metabolites

Organism	Strain or test system	Endpoint	Test substance concentrations	Metabolic activation[a]		Reference
				+S9	–S9	
Rat	F344/bladder epithelium	DNA damage, alkaline elution assay	1,000 or 2,000 ppm, sodium salt in diet for 3 months; no damage at 250 or 500 ppm	+		Morimoto et al. (1989)
Mouse	CD-1/stomach, colon, liver, kidney, bladder, lung	DNA damage, Comet assay	10–2,000 mg/kg	+		Sasaki et al. (2002)
Mouse	CD-1/brain, bone marrow	DNA damage, Comet assay	10–2,000 mg/kg	–		Sasaki et al. (2002)
Mouse	CD-1/stomach, liver, kidney, bladder, lung	DNA damage, Comet assay	2,000 mg/kg	+		Sasaki et al. (1997)
Mouse	CD-1/brain, bone marrow	DNA damage, Comet assay	2,000 mg/kg	–		Sasaki et al. (1997)
Mouse	CD-1/skin	DNA adduct, [^{32}P]-post labeling method	10 or 20 mg applied to skin	+		Pathak and Roy (1993)
Rat	F344/bladder epithelium	DNA adduct, [^{32}P]-post labeling method Cell proliferation	800–12,500 ppm in diet	– +		Smith et al. (1998)
Rat	F344/bladder epithelium	DNA binding	15–1,000 mg/kg by gavage, labeled with [^{14}C]-2-hydroxy-biphenyl, uniformly labeled in phenol ring	–		Kwok et al. (1999)
Silkworm		Mutation	Not specified	–		Kawachi et al. (1980)
4-Hydroxybiphenyl in vitro tests						
S. typhimurium	TA98 TA1535	Mutation	5–1,000 µg/plate 1,000 µg/plate[c]	+ –	NT NT	Narbonne et al. (1987)
	TA1535, 1536, 1537-1, 1538-1		Units provided in Japanese	–		Hanada (1977)
B. subtilis	H17 (rec$^+$) M45 (rec$^-$)	Rec assay	Units provided in Japanese	–	–	Hanada (1977)
2,5-Dihydroxybiphenyl in vitro or in vivo tests						
Human	DNA fragments from plasmid pbcNI	DNA damage, Comet assay	0.1 mM	NT	+[d]	Inoue et al. (1990)
Rat	F344/bladder epithelium	DNA damage, alkaline elution assay	0.05% injected intravesically into bladder wall	–[e]		Morimoto et al. (1989)

Table B-3. Genotoxicity test results for biphenyl metabolites

Organism	Strain or test system	Endpoint	Test substance concentrations	Metabolic activation[a]		Reference
				+S9	–S9	
Mouse	CD-1/skin	DNA adduct, [^{32}P]-post labeling method	10 or 20 mg applied to skin	+		Pathak and Roy (1993)

[a]Lowest concentration resulting in cytotoxicity.
[b]Metabolic activation system derived from rat skin homogenate.
[c]Lowest concentration resulting in precipitation.
[d]Positive response only in the presence of Cu(II)
[e]Injection with 0.05% or 0.1% phenylbenzoquinone, a metabolite of 2,5-dihydroxybiphenyl, produced DNA damage at concentrations of 0.05 or 0.1%, but not at 0.005 or 0.0005%.

NT = not tested; +/– = weakly positive or equivocal result; empty cell = no information available

In bacterial mutagenicity tests or in vitro mammalian tests of 2-hydroxybiphenyl, results were mostly negative or equivocal, but other tests with bacterial systems suggest that oxidative DNA damage following metabolism of 2-hydroxybiphenyl to 2,5-dihydroxybiphenyl is possible (see Table B-3 for references). 2-Hydroxybiphenyl induced DNA repair in *E. coli* strains both with and without S9 (Nishioka and Ogasawara, 1978). Tani et al. (2007) provided evidence that redox cycling of a semiquinone/quinone pair causes oxidative DNA damage following exposure of a mutant *E. coli* strain (WP2, lacking catalase and superoxide dismutase) to 2-hydroxy-biphenyl: 2-hydroxybiphenyl induced streptomycin resistance mutations in the mutant, but not in the wild type. Exposure of *B. subtilis* to 2-hydroxybiphenyl both with and without S9 in the rec assay yielded positive (Kojima and Hiraga, 1978; Hanada, 1977) and negative (Kawachi et al., 1980) results. 2-Hydroxybiphenyl did not induce CAs without S9 in CHL fibroblasts in one study (Kawachi et al., 1980) or with S9 in other studies of CHL fibroblasts (Ishidate et al., 1984) and CHO cells (Yoshida et al., 1978).

Results from in vivo mammalian genotoxicity test systems provide limited evidence for possible genotoxic actions (DNA damage and micronuclei formation) from 2-hydroxybiphenyl through its metabolites, 2,5-dihydroxybiphenyl and phenylbenzoquinone (Table B-3).

DNA damage was detected by the Comet assay in the urinary bladder of CD-1 mice administered single oral doses of 2,000 mg 2-hydroxybiphenyl/kg, but it is unknown if the damage was due to cytotoxicity, direct reaction of DNA with 2-hydroxybiphenyl or its metabolites, or possible oxidative DNA damage from redox cycling of 2,5-dihydroxybiphenyl (Sasaki et al., 2002; Smith et al., 1998). DNA damage was also detected in the urinary bladder of male or female rats intravesically injected with 0.05 or 0.1% phenylbenzoquinone, but not with injections of 0.05% 2-hydroxybiphenyl or 2,5-dihydroxybiphenyl, although DNA damage was found in urinary bladders from male F344 rats fed the sodium salt of 2-hydroxybiphenyl in

the diet for 3 months at 1,000 or 2,000 ppm, but not at 500 or 250 ppm (Morimoto et al., 1989). Topical application of 10 or 20 mg of the sodium salt of 2-hydroxybiphenyl or 5 mg of 2,5-dihydroxybiphenyl to the skin of female CD-1 mice produced several DNA adducts in the skin that were detected by the [^{32}P]-post labeling technique (Pathak and Roy, 1993). Similar adducts were formed in vitro when DNA was incubated with 2-hydroxybiphenyl (1 mM) in the presence metabolic activation from rat skin homogenates, a CYP system, or a prostaglandin synthase system (Pathak and Roy, 1993). In contrast, Smith et al. (1998), using a similar technique to that used by Pathak and Roy (1993), were unable to detect exposure-related DNA adducts in bladder epithelial tissue from male F344 rats fed 800, 4,000, 8,000, or 12,500 ppm 2-hydroxybiphenyl in the diet for 13 weeks. In this experiment, increased bladder cell epithelium proliferation (i.e., increased BrdU incorporation) was observed at 8,000 and 12,500 ppm, dietary concentrations associated with the development of urinary bladder tumors in chronically exposed rats (Smith et al., 1998). Kwok et al. (1999) found no evidence of binding of radioactivity to DNA extracted from the bladder epithelium of male F344 rats given single gavage doses of [^{14}C]-labeled 2-hydroxybiphenyl at 15, 50, 250, 500, or 1,000 mg/kg, but increased protein binding occurred with increasing doses of 250, 500, and 1,000 mg/kg. Kwok et al. (1999) noted that the increase in protein binding increased with increasing dose levels of 250, 500, and 1,000 mg/kg, in parallel with increasing incidence of bladder epithelial lesions (hyperplasia, papillomas, and carcinomas) in rats chronically exposed to 2-hydroxybiphenyl in the diet at 0, 269, and 531 mg/kg.

Increased micronuclei (about threefold increase over controls) and increased cell proliferation (>200-fold increased incorporation of BrdU in DNA) were found in the bladder epithelium of male F344 rats exposed to 2% (2,000 ppm) 2-hydroxybiphenyl in the diet for 2 weeks, without evidence for hypo- or hyperploidy as assayed by fluorescence in situ hybridization with a DNA probe for rat chromosome 4 (Balakrishnan et al., 2002). Similar exposure to 2% NaCl or 2% 2-hydroxybiphenyl + 2% NaCl, produced about two- or six-fold increases of micronuclei in the bladder epithelium, respectively, but neither treatment stimulated bladder epithelium cell proliferation to the same degree as 2% 2-hydroxybiphenyl in the diet Balakrishnan. 2-Hydroxybiphenyl reportedly did not induce SCEs in the bone marrow of rats, but exposure parameters were not specified in the report by Kawachi et al. (1980). The mechanism of 2-hydroxybiphenyl-induced micronuclei is not understood, but, as discussed by Balakrishan et al. (2002), possible mechanisms include: (1) DNA damage from ROS from redox cycling between 2,5-dihydroxybiphenyl and phenylbenzoquinone, (2) interference of the mitotic spindle through covalent modification of proteins, (3) inhibition of enzymes regulating DNA replication, or (4) micronuclei generation as a secondary response to cytotoxicity or regenerative hyperplasia.

Bacterial mutation assays of the major biphenyl metabolite, 4-hydroxybiphenyl, yielded negative results in all but one case that was accompanied by overt cytotoxicity (Narbonne et al.,

1987). 2,5-Dihydroxybiphenyl (i.e., phenylhydroquinone) caused in vitro damage to human DNA from plasmid pbcNI in the presence of Cu(II) (Inoue et al., 1990), DNA adducts when applied to mouse skin (Pathak and Roy, 1993), but did not cause DNA damage when injected intravesically into the urinary bladder of F344 rats at a concentration of 0.05% (Morimoto et al., 1989).

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE REFERENCE DOSE

Datasets used for modeling incidences of nonneoplastic effects in the urinary tract of male and female F344 rats exposed to biphenyl in the diet for 2 years (Umeda et al., 2002) are shown in Table C-1. Datasets used for modeling body weight data, selected clinical chemistry results, and histopathological kidney effects in male and female BDF_1 mice exposed to biphenyl in the diet for 2 years (Umeda et al., 2005) are shown in Table C-2. The dataset for incidence of litters with fetal skeletal anomalies from Wistar rat dams administered biphenyl by gavage on GDs 6–15 (Khera et al., 1979) is shown in Table C-3.

Table C-1. BMD modeling datasets for incidences of nonneoplastic effects in the urinary tract of male and female F344 rats exposed to biphenyl in the diet for 2 years

	Males (n = 50)				Females (n = 50)			
Biphenyl dietary concentration (ppm)	**0**	**500**	**1,500**	**4,500**	**0**	**500**	**1,500**	**4,500**
TWA body weight (kg)[a]	**0.411**	**0.412**	**0.408**	**0.357**	**0.251**	**0.246**	**0.246**	**0.216**
Calculated dose (mg/kg-d)[b]	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Effect								
Renal pelvis								
Nodular transitional cell hyperplasia	0	1	1	21[c]	0	0	1	12[c]
Simple transitional cell hyperplasia	6	8	5	19[d]	3	5	12[d]	25[c]
Mineralization	9	6	10	18[e]	12	12	18	27[d]
Other kidney effects								
Hemosiderin deposit[f]	0	0	0	0	4	8	22[c]	25[c]
Papillary mineralization	9	9	14	23[d]	2	6	3	12[c]
Bladder								
Combined transitional cell hyperplasia[g]	0	0	0	45	1	0	1	10

[a]TWA body weight calculated using graphically-presented body weight data from Umeda et al. (2002).
[b]Calculated doses based on TWA body weights and chronic reference food consumption values for F344 rats (0.030 kg/day for males and 0.021 kg/day for females) (U.S. EPA, 1988, Table 1-6).
[c]Significantly different from control group ($p < 0.01$) according to χ^2 test.
[d]Significantly different from control group ($p < 0.05$) according to χ^2 test.
[e]Significantly different from controls ($p < 0.05$) according to Fisher's exact test.
[f]Male data for incidences of hemosiderin deposits not selected for quantitative analysis..
[g]Female data for incidences of combined transitional cell hyperplasia not selected for quantitative analysis.

Source: Umeda et al. (2002).

Table C-2. BMD modeling datasets for body weight, selected clinical chemistry results, and histopathological kidney effects in male and female BDF_1 mice exposed to biphenyl in the diet for 2 years

Endpoint	Biphenyl concentration in the diet (ppm)			
	0	**667**	**2,000**	**6,000**
Males				
Dose (mg/kg-d)	**0**	**97**	**291**	**1,050**
Histopathological kidney effect	n = 50	n = 49	n = 50	n = 50
Mineralization inner stripe-outer medulla	9	8	14	14
Clinical chemistry parameter	n = 34	n = 39	n = 37	n = 37
BUN (mg/dL)	20.2 ± 3.6	22.0 ± 4.0	23.2 ± 4.4[a]	22.9 ± 2.7[b]
Body weight	n = 35	n = 41	n = 41	n = 39
Mean terminal body weight (g)	46.9 ± 4.9	43.1 ± 7.9	42.9 ± 6.0[a]	32.4 ± 3.6[b]
Females				
Dose (mg/kg-d)	**0**	**134**	**414**	**1,420**
Histopathological kidney effect	n = 50	n = 50	n = 50	n = 49
Mineralization inner stripe-outer medulla	3	5	12[c]	26[d]
Clinical chemistry parameter	n = 28	n = 20	n = 22	n = 31
AST (IU/L)	75 ± 27	120 ± 110	211 ± 373[b]	325 ± 448[b]
ALT (IU/L)	32 ± 18	56 ± 46	134 ± 231[b]	206 ± 280[b]
AP (IU/L)	242 ± 90	256 ± 121	428 ± 499	556 ± 228[b]
LDH (IU/L)	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161[a]
BUN (mg/dL)	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7[b]
Body weight	n = 31	n = 22	n = 25	n = 32
Mean terminal body weight (g)	34.0 ± 4.0	32.5 ± 3.3	30.5 ± 3.1[b]	25.5 ± 3.0[b]

[a]Significantly different from controls ($p < 0.05$) according to Dunnett's test.
[b]Significantly different from controls ($p < 0.01$) according to Dunnett's test.
[c]Significantly different from controls ($p < 0.05$) according to Fisher's exact test.
[d]Significantly different from controls ($p < 0.01$) according to Fisher's exact test.

Source: Umeda et al. (2005).

Table C-3. BMD modeling dataset for incidence of litters with fetal skeletal anomalies from Wistar rat dams administered biphenyl by gavage on GDs 6–15

Effect	Dose (mg/kg-d)				
	0	125	250	500	1,000
Litters with fetal skeletal anomalies[a]/litters examined	8/16	11/20	13/18	15/18[b]	6/9

[a]The study authors reported one runted fetus in the control group and one fetus with kinky tail in the 250 mg/kg-day dose group, which may have influenced the reported incidence data for anomalous litters/litters examined.
[b]Significantly different from controls ($p < 0.05$) according to Fisher's exact test conducted for this review.

Source: Khera et al. (1979).

Goodness-of-fit statistics and benchmark results for each of the modeled biphenyl-induced nonneoplastic effects from the chronically-exposed rats (Umeda et al., 2002) and mice (Umeda et al., 2005) and the gestationally-exposed rats (Khera et al., 1979) are summarized in Tables C-4 through C-24. Each table of modeled results for a particular effect is followed by the information from the output file of the best-fitting model for that effect.

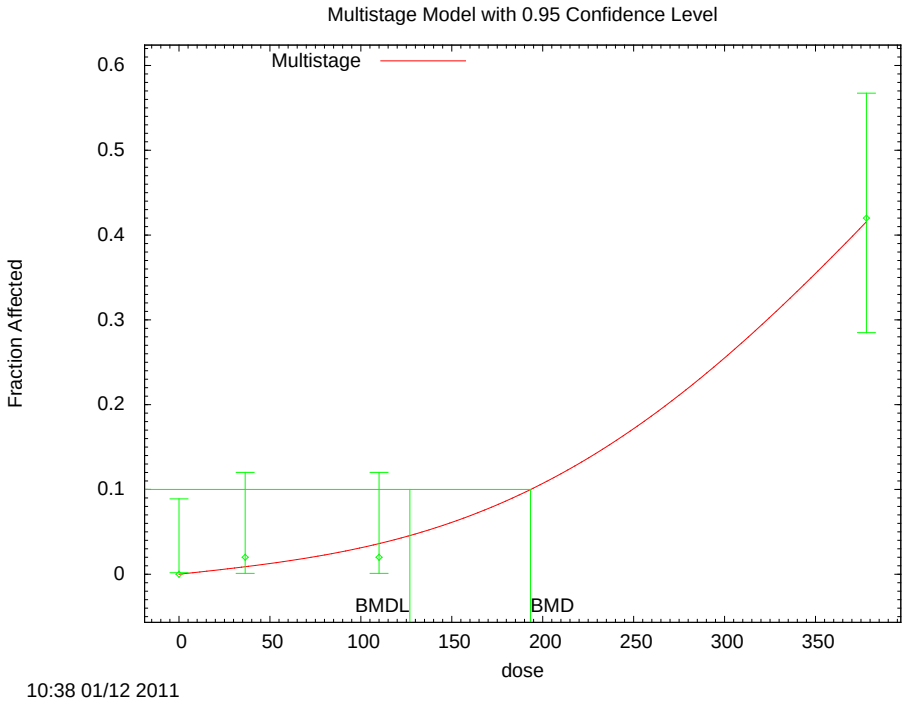
Table C-4. Summary of BMD modeling results for incidence of renal nodular transitional cell hyperplasia in male F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b]	0.31	0.73	95.02	169.71	74.44	212.00	120.62
Logistic	0.64	0.74	92.72	178.92	133.35	233.81	192.35
Log-Logistic[b]	0.31	0.74	95.01	172.40	75.93	216.08	120.70
Log-Probit[b]	0.31	0.71	95.03	163.38	89.50	202.25	128.71
Multistage (3-degree)[c,d]	**0.58**	**0.84**	**92.60**	**133.82**	**69.08**	**193.30**	**126.95**
Probit	0.59	0.84	92.76	157.59	117.53	212.09	173.76
Weibull[b]	0.31	0.75	95.00	175.08	73.08	221.75	121.01

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.
[d]Betas restricted to ≥0.

BMD = maximum likelihood estimate of the dose associated with the selected ; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
          Multistage Model. (Version: 3.2;  Date: 05/26/2010)
          Input Data File:
C:\USEPA\IRIS\biphenyl\rat\renalnodularhyper\male\mst_nodhypMrev_MS_3.(d)
          Gnuplot Plotting File:
C:\USEPA\IRIS\biphenyl\rat\renalnodularhyper\male\mst_nodhypMrev_MS_3.plt
                                                  Wed Jan 12 10:38:57 2011
 =================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1-beta2*dose^2-beta3*dose^3)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3
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 =   0.00721859
                        Beta(1) = 3.68302e-005
                        Beta(2) =            0
                        Beta(3) = 9.69211e-009

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -Background    -Beta(2) have been estimated at a boundary point, or
have been specified by the user, and do not appear in the correlation matrix )
                Beta(1)      Beta(3)
   Beta(1)            1        -0.95
   Beta(3)        -0.95            1

                                Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background                0           *                *                   *
```

```
       Beta(1)      0.000234424           *               *                 *
       Beta(2)                0           *               *                 *
       Beta(3)     8.31393e-009           *               *                 *
* - Indicates that this value is not calculated.

                      Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -43.8185         4
   Fitted model        -44.3014         2      0.965856      2           0.617
  Reduced model        -71.3686         1       55.1002      3         <.0001

          AIC:         92.6029

                                Goodness  of  Fit
                                                                      Scaled
    Dose      Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          50        0.000
   36.4000     0.0089         0.445     1.000          50        0.836
  110.0000     0.0362         1.809     1.000          50       -0.613
  378.0000     0.4159        20.794    21.000          50        0.059

 Chi^2 = 1.08      d.f. = 2        P-value = 0.5832

   Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        193.298
            BMDL =        126.946
            BMDU =        248.35
Taken together, (126.946, 248.35 ) is a 90% two-sided confidence interval for the BMD
```

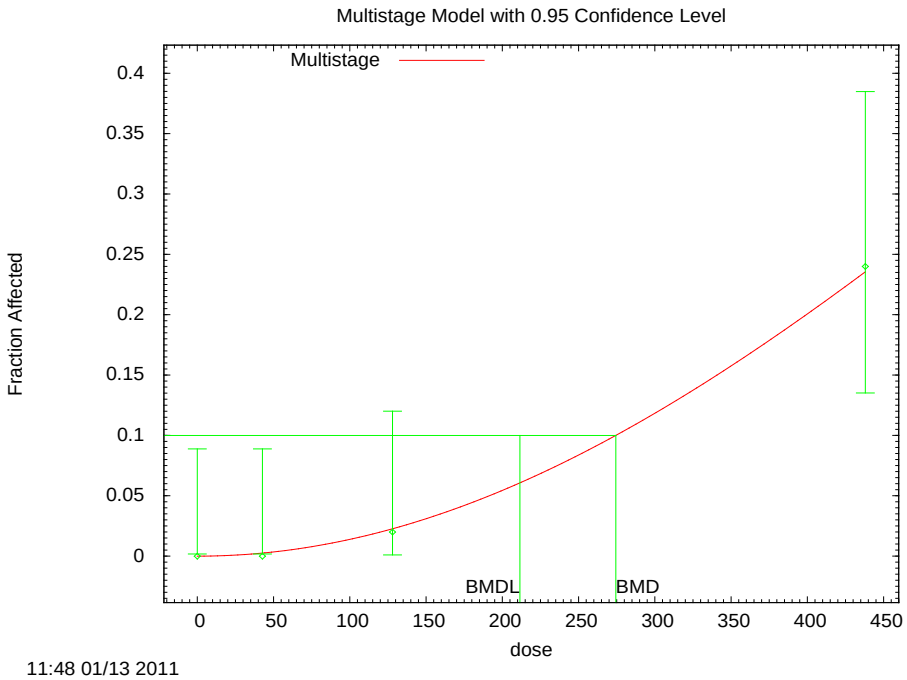
Table C-5. Summary of BMD modeling results for incidence of renal nodular transitional cell hyperplasia in female F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b]	0.96	-0.24	69.04	200.54	118.95	276.46	198.73
Logistic	0.69	0.63	69.93	277.38	211.02	343.52	289.03
Log-Logistic[b]	0.96	-0.26	69.07	203.45	118.10	279.78	196.91
Log-Probit[b]	0.99	-0.15	68.96	188.92	134.61	261.35	193.58
Multistage (2-degree)[c,d]	**0.99**	**-0.36**	**67.19**	**191.47**	**121.69**	**274.42**	**211.52**
Probit	0.76	0.54	69.69	253.65	190.94	324.08	268.17
Weibull[b]	0.95	-0.27	69.08	207.16	119.11	285.37	201.63

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0.
[d]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
         Multistage Model. (Version: 3.2;  Date: 05/26/2010)
         Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalnodularhyper/female/mst_nodhypFrev_MS_2.(d)
         Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalnodularhyper/female/mst_nodhypFrev_MS_2.plt
                                                  Thu Jan 13 11:48:49 2011
 =================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1-beta2*dose^2)]
The parameter betas are restricted to be positive
Dependent variable = incidence
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
                        Beta(1) =           0


           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -Background    -Beta(1) have been estimated at a boundary point, or
have been specified by the user, and do not appear in the correlation matrix )
               Beta(2)
   Beta(2)           1

                                Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background                0           *                 *                   *
       Beta(1)                0           *                 *                   *
       Beta(2)     1.39908e-006           *                 *                   *
* - Indicates that this value is not calculated.
```

```
                    Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -32.456         4
   Fitted model        -32.5947         1     0.277585      3          0.9642
  Reduced model        -48.1018         1      31.2917      3         <.0001

          AIC:         67.1895

                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          50         0.000
   42.7000     0.0025         0.127     0.000          50        -0.357
  128.0000     0.0227         1.133     1.000          50        -0.126
  438.0000     0.2354        11.770    12.000          50         0.077

 Chi^2 = 0.15      d.f. = 3        P-value = 0.9853

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        274.422
            BMDL =        211.518
            BMDU =        351.444
Taken together, (211.518, 351.444) is a 90% two-sided confidence interval for the BMD
```

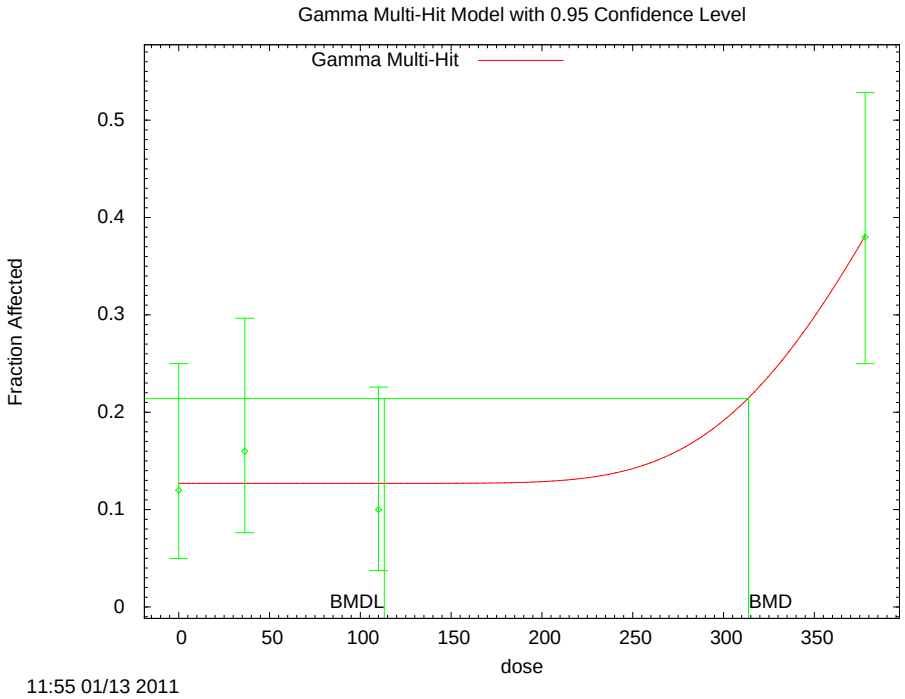
Table C-6. Summary of BMD modeling results for incidence of renal simple transitional cell hyperplasia in male F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b,c]	**0.66**	**0.71**	**184.41**	**284.70**	**55.27**	**313.76**	**113.22**
Logistic	0.35	-1.18	185.78	96.07	73.33	171.37	131.76
Log-Logistic[b]	0.36	0.71	186.41	320.26	58.80	340.21	115.09
Log-Probit[b]	0.36	0.71	186.41	284.12	100.23	312.44	144.14
Multistage (3-degree)[d]	0.60	0.74	184.59	201.02	52.30	255.53	107.40
Probit	0.33	-1.22	185.92	90.26	68.00	164.29	124.13
Weibull[b]	0.36	0.71	186.41	324.89	55.27	344.08	113.14

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit differed by less than threefold.
[d]Betas restricted to ≥0.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
================================================================
          Gamma Model. (Version: 2.15;  Date: 10/28/2009)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalsimplehyper/male/gam_rensimphypMrev_gamma.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalsimplehyper/male/gam_rensimphypMrev_gamma.plt
                                                  Thu Jan 13 11:55:07 2011
 ================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response]= background+(1-
background)*CumGamma[slope*dose,power], where CumGamma(.) is the cummulative Gamma distribution
function
Dependent variable = incidence
Independent variable = dose
Power parameter is restricted as power >=1
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.134615
                          Slope =   0.00398471
                          Power =      2.55235

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -Power have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
              Background        Slope
Background             1        -0.27
     Slope         -0.27            1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background         0.126666        0.0271566           0.0734404            0.179892
          Slope        0.0408652       0.00241924           0.0361236           0.0456068
          Power               18               NA
NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
has no standard error.
```

```
                    Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -89.7871         4
   Fitted model        -90.2033         2      0.832451      2          0.6595
  Reduced model        -97.2446         1        14.915      3        0.001891

          AIC:         184.407

                              Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------------
    0.0000     0.1267         6.333     6.000          50       -0.142
   36.4000     0.1267         6.333     8.000          50        0.709
  110.0000     0.1267         6.333     5.000          50       -0.567
  378.0000     0.3800        19.000    19.000          50        0.000

 Chi^2 = 0.84      d.f. = 2        P-value = 0.6558

Benchmark Dose Computation
Specified effect =         0.1
Risk Type        =      Extra risk
Confidence level =         0.95
             BMD =       313.755
            BMDL =       113.219
```

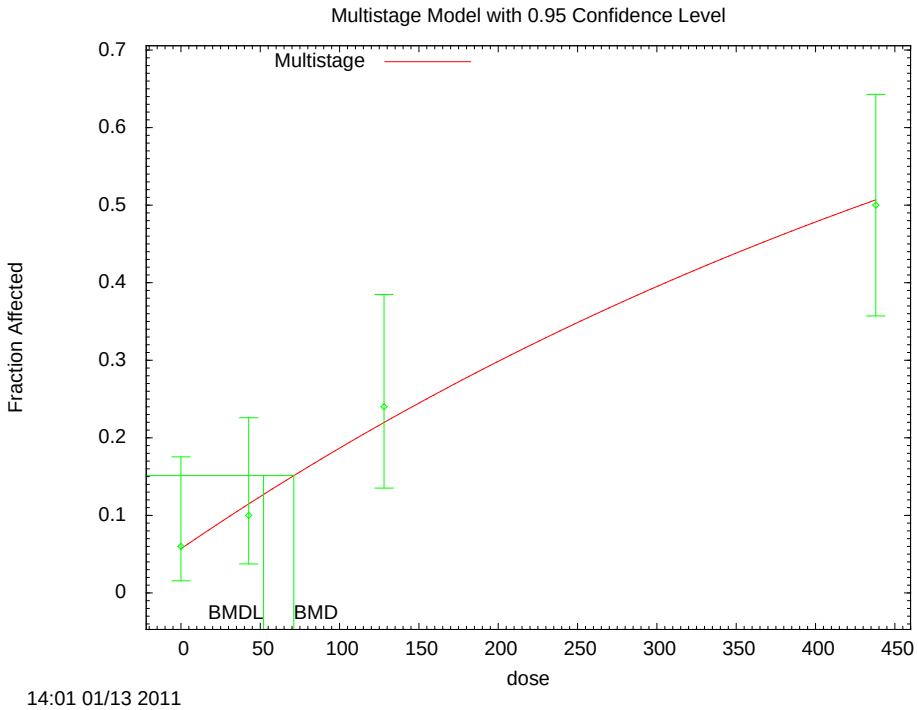
Table C-7. Summary of BMD modeling results for incidence of renal simple transitional cell hyperplasia in female F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b], Weibull[b], Multistage (1-degree)[c,d]	**0.89**	**0.34**	**183.87**	**34.63**	**25.35**	**71.12**	**52.08**
Logistic	0.28	1.29	186.14	83.08	66.43	145.87	119.22
Log-Logistic[b]	0.71	-0.26	185.77	37.52	18.90	71.51	39.91
Log-Probit[b]	0.41	1.00	185.39	84.12	62.52	120.97	89.91
Probit	0.33	1.22	185.77	75.68	60.94	135.30	110.85

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Selected model; the gamma and Weibull models took the form of a 1-degree polynomial multistage model and produced identical goodness of fit statistics and BMD values; the model with the lowest AIC was selected because BMDL values for models providing adequate fit differed by less than threefold.
[d]Betas restricted to ≥0.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
          Multistage Model. (Version: 3.2;  Date: 05/26/2010)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalsimplehyper/female/mst_simplehypFrev_MS_1.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalsimplehyper/female/mst_simplehypFrev_MS_1.plt
                                                  Thu Jan 13 14:01:13 2011
 =================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                    Background =    0.0607741
                       Beta(1) =   0.00145231

           Asymptotic Correlation Matrix of Parameter Estimates
             Background      Beta(1)
Background            1        -0.61
   Beta(1)        -0.61            1

                                Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background         0.057038            *                *                   *
       Beta(1)       0.00148135            *                *                   *
* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -89.8139         4
```

```
   Fitted model        -89.9369         2      0.246113      2          0.8842
  Reduced model        -106.633         1       33.6378      3         <.0001

           AIC:         183.874

                                  Goodness  of  Fit
                                                                        Scaled
     Dose      Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0570         2.852     3.000          50        0.090
   42.7000     0.1148         5.742     5.000          50       -0.329
  128.0000     0.2199        10.995    12.000          50        0.343
  438.0000     0.5072        25.358    25.000          50       -0.101

 Chi^2 = 0.24      d.f. = 2        P-value = 0.8850

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =         71.1248
            BMDL =         52.0766
            BMDU =        105.072
Taken together, (52.0766, 105.072) is a 90% two-sided confidence interval for the BMD
```

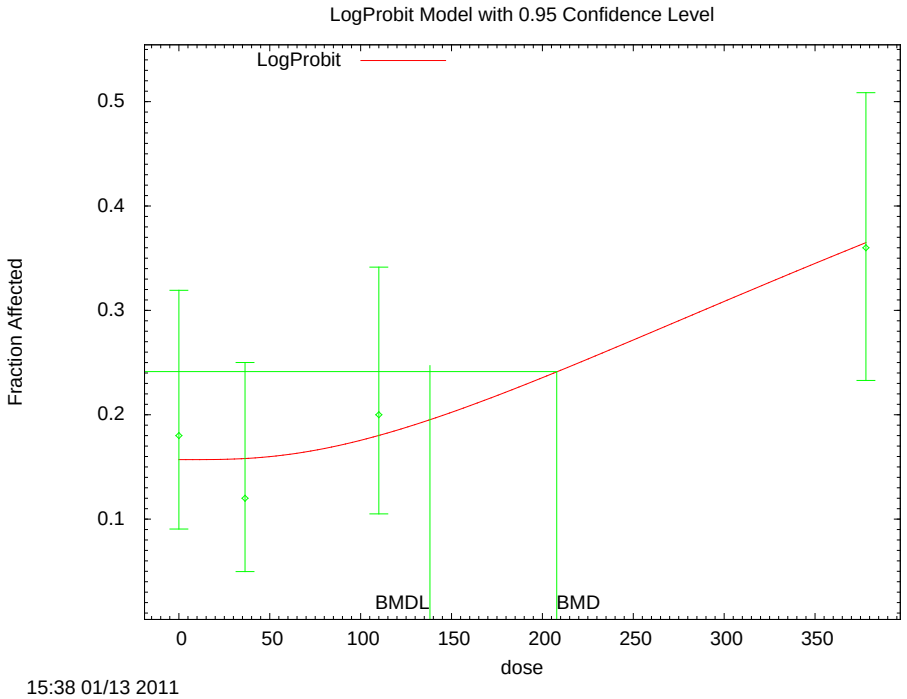
Table C-8. Summary of BMD modeling results for incidence of mineralization in renal pelvis of male F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b]	0.35	-0.75	206.13	130.11	42.91	201.71	88.15
Logistic	0.58	-0.79	204.33	98.62	70.79	181.36	130.04
Log-Logistic[b]	0.34	-0.75	206.14	128.13	36.96	199.42	78.03
Log-Probit[b,c]	**0.64**	**-0.74**	**204.13**	**144.55**	**96.05**	**207.88**	**138.13**
Multistage (1-degree)[d]	0.51	-0.84	204.60	70.84	41.20	145.51	84.62
Probit	0.57	-0.80	204.35	94.16	66.44	175.86	123.70
Weibull[b]	0.34	-0.75	206.15	131.37	42.84	205.20	88.00

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.
[d]Betas restricted to ≥0.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
         Probit Model. (Version: 3.2;  Date: 10/28/2009)
         Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalmineral/male/lnp_minpelvMrev_logprobit.(d)
         Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalmineral/male/lnp_minpelvMrev_logprobit.plt
                                                  Thu Jan 13 15:38:28 2011
=================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = Background + (1-Background) *
CumNorm(Intercept+Slope*Log(Dose)), where CumNorm(.) is the cumulative normal distribution
function
Dependent variable = incidence
Independent variable = dose
Slope parameter is restricted as slope >= 1
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
User has chosen the log transformed model

                 Default Initial (and Specified) Parameter Values
                    background =         0.18
                     intercept =     -6.59931
                         slope =            1

          Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -slope have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
             background    intercept
background            1        -0.46
intercept         -0.46            1

                              Parameter Estimates
                                                    95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background         0.157045        0.0325697          0.0932095             0.22088
     intercept         -6.61851         0.281947           -7.17111            -6.0659
         slope                1               NA
NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
has no standard error.
```

```
                    Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model         -99.607         4
   Fitted model       -100.063         2       0.91202      2          0.6338
  Reduced model       -104.101         1       8.98864      3         0.02944

          AIC:         204.126

                              Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------------
    0.0000     0.1570         7.852     9.000          50        0.446
   36.4000     0.1581         7.905     6.000          50       -0.738
  110.0000     0.1803         9.014    10.000          50        0.363
  378.0000     0.3653        18.267    18.000          50       -0.079

 Chi^2 = 0.88      d.f. = 2        P-value = 0.6434

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        207.879
            BMDL =        138.127
```

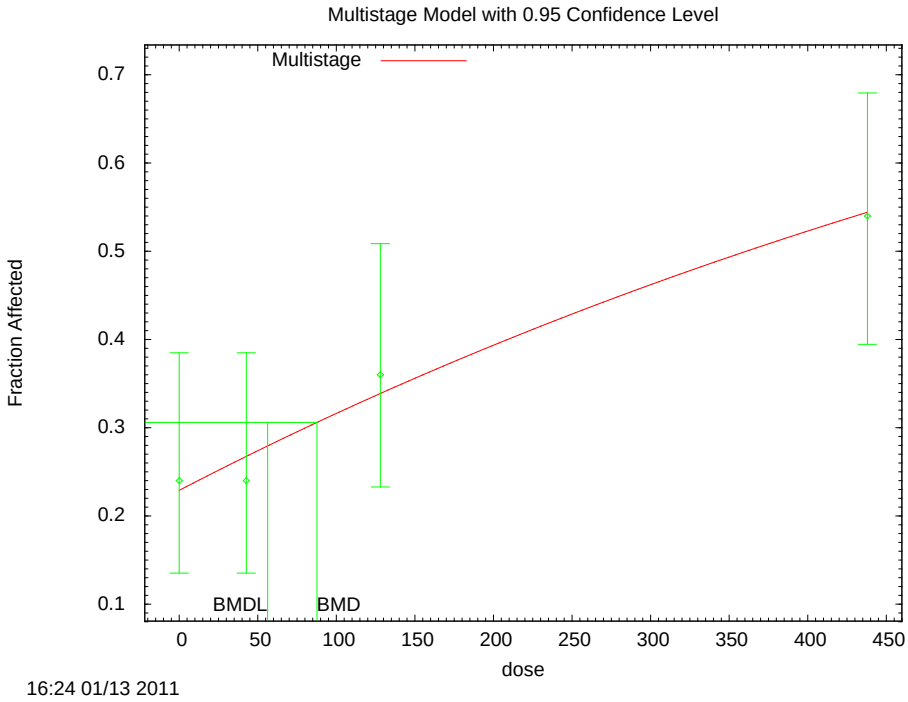
Table C-9. Summary of BMD modeling results for incidence of mineralization in renal pelvis of female F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b]	0.57	-0.43	250.89	44.66	27.40	90.32	56.28
Logistic	0.76	0.59	249.10	64.48	48.11	123.84	92.31
Log-Logistic[b]	<0.001	2.90	263.72	1.33×10^{15}	NA	1.58×10^{15}	NA
Log-Probit[b]	<0.001	2.90	263.72	1.54×10^{14}	NA	2.21×10^{14}	NA
Multistage (1-degree)[c,d]	**0.85**	**-0.44**	**248.89**	**42.68**	**27.40**	**87.67**	**56.28**
Probit	0.77	0.57	249.08	62.20	46.34	120.41	89.56
Weibull[b]	0.56	-0.44	250.89	43.32	27.40	88.56	56.28

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0.
[d]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
         Multistage Model. (Version: 3.2;  Date: 05/26/2010)
         Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalmineral/female/mst_minpelvlFrev_MS_1.(d)
         Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/renalmineral/female/mst_minpelvlFrev_MS_1.plt
                                                  Thu Jan 13 16:24:18 2011
 =================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                    Background =     0.230737
                       Beta(1) =   0.00118679

           Asymptotic Correlation Matrix of Parameter Estimates
             Background      Beta(1)
Background            1        -0.62
   Beta(1)        -0.62            1

                               Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background         0.228898            *                *                   *
       Beta(1)        0.0012018            *                *                   *
* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

```
    Full model       -122.276         4
  Fitted model       -122.443         2      0.334544      2          0.846
 Reduced model       -128.859         1       13.1664      3        0.00429

          AIC:         248.887

                                Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------
    0.0000     0.2289        11.445    12.000          50         0.187
   42.7000     0.2675        13.374    12.000          50        -0.439
  128.0000     0.3388        16.942    18.000          50         0.316
  438.0000     0.5445        27.224    27.000          50        -0.064

 Chi^2 = 0.33      d.f. = 2        P-value = 0.8473

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =         87.669
            BMDL =         56.2773
            BMDU =        172.188
Taken together, (56.2773, 172.188) is a 90% two-sided confidence
```

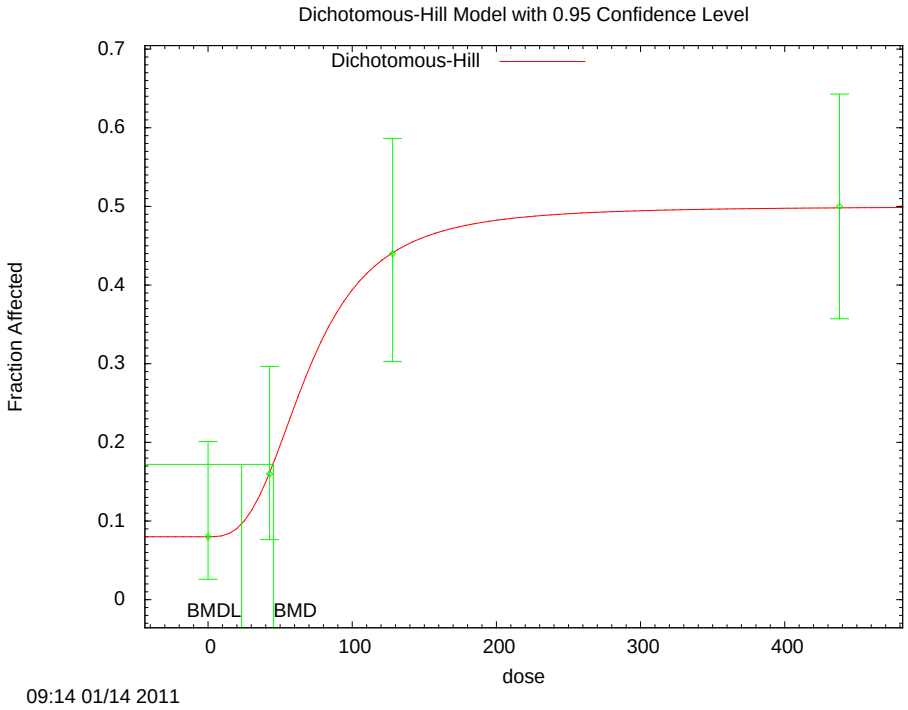
Table C-10. Summary of BMD modeling results for incidence of hemosiderin deposits in the kidney of female F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b], Weibull[b], Multistage (1-degree)[c]	0.022	2.36	220.99	29.64	21.20	60.87	43.54
Logistic	0.002	2.92	225.98	66.06	52.04	123.37	97.71
Log-Logistic[b]	0.093	1.75	218.35	19.21	12.74	40.56	26.89
Log-Probit[b]	0.002	2.82	225.97	74.77	52.43	107.53	75.40
Probit	0.002	2.90	225.57	61.90	49.07	116.90	92.96
Dichotomous-Hill[d,e]	**0.9997**	**0.026**	**213.75**	**34.28**	**12.76**	**45.32**	**23.29**

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0.
[d]Selected model; the only model with an adequate fit (χ^2 *p*-value > 0.1).
[e]v = 0.5 (specified), g = 0.16 (specified), intercept = 0.08 (initialized), slope = 1 (initialized).

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=============================================================================
          Dichotomous Hill Model. (Version: 1.2; Date: 12/11/2009)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/hemosiderin/female/dhl_hemosidFrev_dichotomous
hill.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/hemosiderin/female/dhl_hemosidFrev_dichotomous
hill.plt
                                                        Fri Jan 14 09:14:35 2011
 =============================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = v*g +(v-v*g)/[1+EXP(-intercept-
slope*Log(dose))] where: 0 <= g < 1, 0 < v <= 1v is the maximum probability of response predicted
by the model, and v*g is the background estimate of that probability.
Dependent variable = incidence
Independent variable = dose
Parameter v is set to 0.5
Parameter g is set to 0.16
Slope parameter is restricted as slope >= 1
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                 User Inputs Initial Parameter Values
                             v =        -9999   Specified
                             g =        -9999   Specified
                     intercept =         0.08
                         slope =            1

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -v    -g have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
              intercept        slope
intercept             1        -0.99
     slope        -0.99            1

                                  Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
```

```
     intercept         -12.5334          5.83724           -23.9742           -1.09265
         slope          2.95297          1.43635           0.137773            5.76817

                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -104.876         4
   Fitted model        -104.876         2   0.000679954      2          0.9997
  Reduced model        -121.314         1       32.8756      3         <.0001

           AIC:         213.752

                                 Goodness  of  Fit
                                                                        Scaled
     Dose      Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0800         4.000     4.000          50        0.000
   42.7000     0.1600         7.998     8.000          50        0.001
  128.0000     0.4401        22.007    22.000          50       -0.002
  438.0000     0.4982        24.908    25.000          50        0.026

 Chi^2 = 0.00      d.f. = 2        P-value = 0.9997

Benchmark Dose Computation
Specified effect =         0.1
Risk Type        =      Extra risk
Confidence level =        0.95
             BMD =       45.3249
            BMDL =       23.2881
```

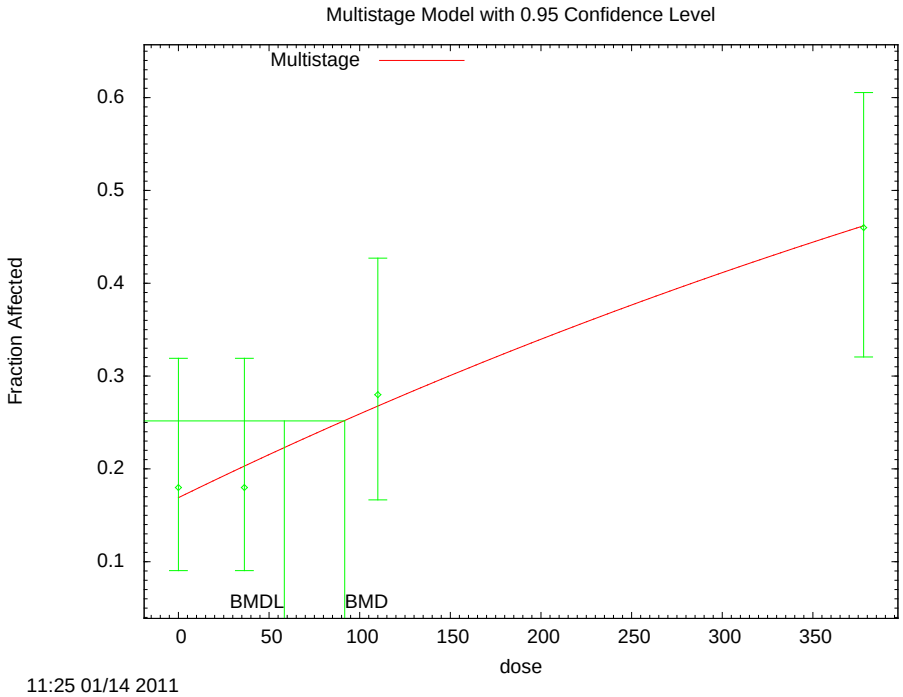
Table C-11. Summary of BMD modeling results for incidence of papillary mineralization in the kidney of male F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b]	0.63	-0.37	228.81	51.08	28.48	99.83	58.49
Logistic	0.81	0.51	226.99	70.07	52.70	131.45	98.95
Log-Logistic[b]	<0.001	2.93	241.27	5.64×10^{14}	NA	6.68×10^{14}	NA
Log-Probit[b]	0.001	2.93	239.27	5.13×10^{13}	NA	7.38×10^{13}	NA
Multistage (1-degree)[c,d]	**0.88**	**-0.40**	**226.82**	**44.66**	**28.45**	**91.74**	**58.44**
Probit	0.82	0.48	226.96	66.59	49.79	126.42	94.42
Weibull[b]	0.63	-0.37	228.81	49.89	28.47	98.66	58.48

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0.
[d]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
          Multistage Model. (Version: 3.2;  Date: 05/26/2010)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/pappmineral/male/mst_papminMrev_MS_1.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/pappmineral/male/mst_papminMrev_MS_1.plt
                                                  Fri Jan 14 11:25:01 2011
 =================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                    Background =     0.168963
                       Beta(1) =   0.00114658

           Asymptotic Correlation Matrix of Parameter Estimates
             Background      Beta(1)
Background            1        -0.62
   Beta(1)        -0.62            1

                               Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background         0.168634            *               *                   *
       Beta(1)       0.00114846            *               *                   *
* - Indicates that this value is not calculated.

                       Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -111.284         4
```

```
   Fitted model        -111.409         2      0.250221      2          0.8824
  Reduced model        -117.634         1       12.6991      3        0.005335

           AIC:         226.819

                                Goodness  of  Fit
                                                                      Scaled
     Dose      Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------------
    0.0000      0.1686         8.432      9.000          50         0.215
   36.4000      0.2027        10.134      9.000          50        -0.399
  110.0000      0.2673        13.365     14.000          50         0.203
  378.0000      0.4614        23.071     23.000          50        -0.020

 Chi^2 = 0.25      d.f. = 2        P-value = 0.8839

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =         91.741
            BMDL =         58.4361
            BMDU =        182.915
Taken together, (58.4361, 182.915) is a 90% two-sided confidence interval for the BMD
```

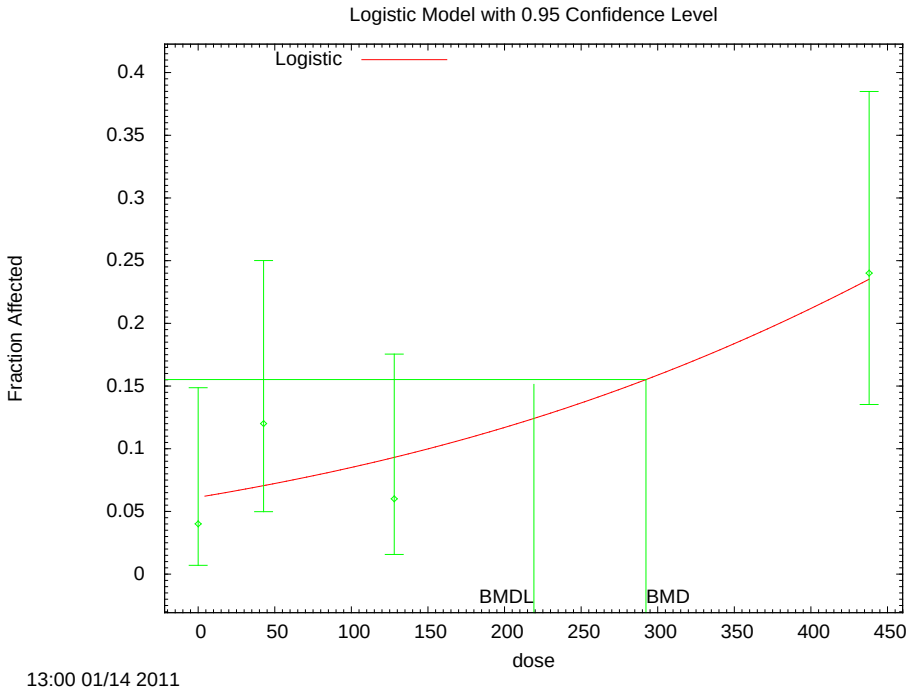
Table C-12. Summary of BMD modeling results for incidence of papillary mineralization in the kidney of female F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b]	0.11	1.27	139.76	360.00	68.91	397.57	141.55
Logistic[c]	**0.23**	**1.37**	**138.04**	**175.24**	**129.91**	**292.33**	**219.17**
Log-Logistic[b]	0.11	1.27	139.76	388.83	61.62	413.84	130.08
Log-Probit[b]	0.11	1.27	139.76	356.94	150.95	395.27	217.08
Multistage (1-degree)[d]	0.21	1.28	138.38	113.15	65.01	232.43	133.53
Probit	0.23	1.36	138.08	164.88	119.64	282.98	206.34
Weibull[b]	0.11	1.27	139.76	391.23	68.91	415.47	141.55

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.
[d]Betas restricted to ≥0.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
================================================================
         Logistic Model. (Version: 2.13; Date: 10/28/2009)
         Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/pappmineral/female/log_papmineralFrev_logistic.(d)
         Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/pappmineral/female/log_papmineralFrev_logistic.plt
                                                  Fri Jan 14 13:00:44 2011
 ================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = 1/[1+EXP(-intercept-slope*dose)]
Dependent variable = incidence
Independent variable = dose
Slope parameter is not restricted
Total number of observations = 4
Total number of records with missing values = 0
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   Specified
                      intercept =     -2.67819
                          slope =   0.00343504

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -background have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
               intercept        slope
intercept            1        -0.78
     slope        -0.78            1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     intercept         -2.72974         0.364791          -3.44472           -2.01477
         slope       0.00353956       0.00119641         0.00119464         0.00588449

                     Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -65.6458         4
   Fitted model        -67.0198         2       2.74796      2          0.2531
  Reduced model        -71.3686         1       11.4455      3        0.009545
```

```
          AIC:          138.04

                              Goodness  of  Fit
                                                                      Scaled
    Dose      Est._Prob.     Expected     Observed      Size        Residual
  ----------------------------------------------------------------------------
    0.0000      0.0612         3.062      2.000          50        -0.626
   42.7000      0.0705         3.526      6.000          50         1.366
  128.0000      0.0931         4.654      3.000          50        -0.805
  438.0000      0.2352        11.758     12.000          50         0.081

 Chi^2 = 2.91      d.f. = 2        P-value = 0.2330

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        292.331
            BMDL =        219.166
```

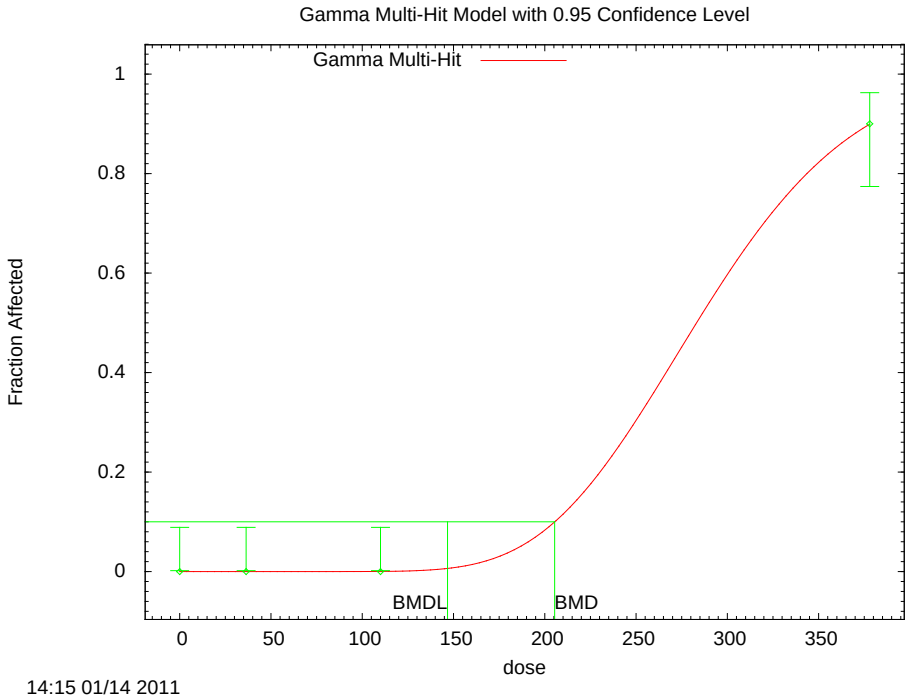
Table C-13. Summary of BMD modeling results for incidence of combined transitional cell hyperplasia in the bladder of male F344 rats exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b,c]	**1.00**	**-0.12**	**34.54**	**186.38**	**125.23**	**205.40**	**146.73**
Logistic	1.00	0.00	36.51	314.74	151.02	323.93	182.76
Log-Logistic[b]	1.00	0.00	36.51	283.35	126.46	295.47	147.96
Log-Probit[b]	1.00	0.00	36.51	227.03	122.78	241.87	140.96
Multistage (3-degree)[d]	0.39	-1.63	40.12	109.67	93.51	139.41	123.14
Probit	1.00	0.00	36.51	266.72	137.23	280.54	166.54
Weibull[b]	1.00	0.00	36.51	300.36	131.93	313.72	160.88

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.
[d]Betas restricted to ≥0.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2002).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
================================================================
         Gamma Model. (Version: 2.15;  Date: 10/28/2009)
         Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/bladdercombinedhyper/male/gam_bladcomhypMrev_gamma.(d
)
         Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/bladdercombinedhyper/male/gam_bladcomhypMrev_gamma.pl
t
                                                    Fri Jan 14 14:15:19 2011
 ================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response]= background+(1-
background)*CumGamma[slope*dose,power], where CumGamma(.) is the cummulative Gamma distribution
function
Dependent variable = incidence
Independent variable = dose
Power parameter is restricted as power >=1
Total number of observations = 4Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =    0.0192308
                          Slope =    0.0320399
                          Power =      8.56462

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -Background    -Power have been estimated at a boundary point, or
have been specified by the user, and do not appear in the correlation matrix )
                  Slope
     Slope            1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0               NA
          Slope        0.0624215       0.00323795          0.0560752           0.0687677
          Power               18               NA
NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
has no standard error.
```

```
                    Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -16.2541         4
    Fitted model       -16.2687         1     0.0290112      3          0.9987
  Reduced model        -106.633         1       180.757      3         <.0001

           AIC:         34.5373

                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          50         0.000
   36.4000     0.0000         0.000     0.000          50        -0.000
  110.0000     0.0003         0.014     0.000          50        -0.120
  378.0000     0.8996        44.981    45.000          50         0.009

 Chi^2 = 0.01      d.f. = 3        P-value = 0.9995

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        205.404
            BMDL =        146.733
```

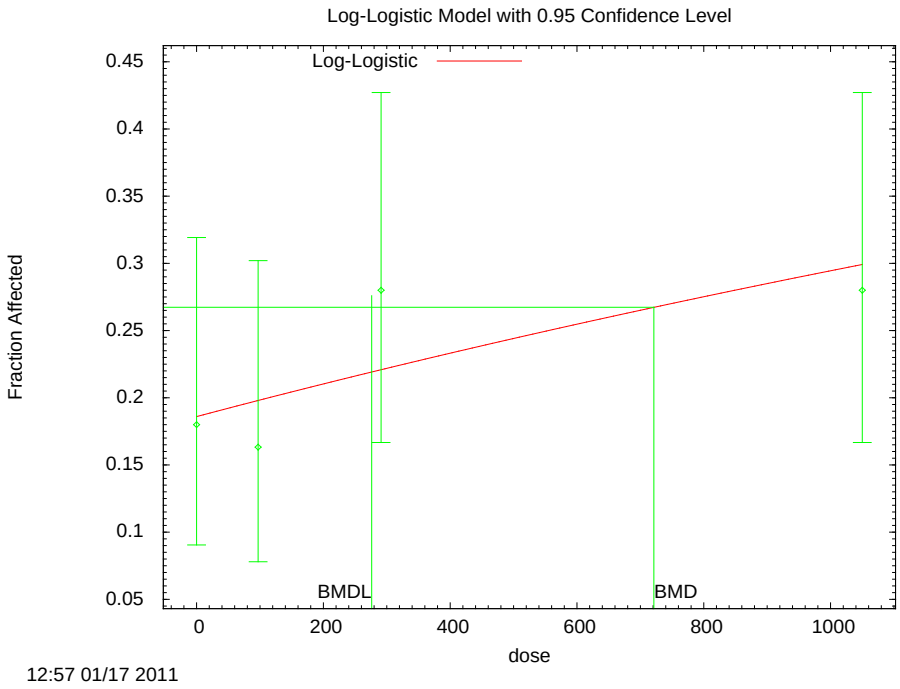
Table C-14. Summary of BMD modeling results for incidence of mineralization in the kidney (inner stripe outer medulla) of male BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b], Weibull[b], Multistage (1-degree)[c]	0.46	1.03	214.84	369.24	155.65	758.45	319.71
Logistic	0.43	1.07	214.97	454.16	238.75	856.07	446.12
Log-Logistic[b,d]	**0.48**	**1.01**	**214.79**	**341.66**	**130.84**	**721.28**	**276.22**
Log-Probit[b]	0.33	1.24	215.51	710.74	377.36	1,022.10	542.66
Probit	0.44	1.07	214.95	442.78	227.50	844.26	430.21

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0.
[d]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2005).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
================================================================
          Logistic Model. (Version: 2.13; Date: 10/28/2009)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/minmedulla/male/lnl_minmedullM_loglogistic.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/minmedulla/male/lnl_minmedullM_loglogistic.plt
                                                  Mon Jan 17 12:57:13 2011
 ================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background+(1-background)/[1+EXP(-
intercept-slope*Log(dose))]
Dependent variable = incidence
Independent variable = dose
Slope parameter is restricted as slope >= 1
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =         0.18
                     intercept =     -8.98323
                         slope =      1.06986

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -slope have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
             background    intercept
background            1        -0.64
intercept         -0.64            1

                              Parameter Estimates
                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.185925            *                 *                   *
      intercept         -8.77824            *                 *                   *
          slope                1            *                 *                   *
* - Indicates that this value is not calculated.

                       Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

```
    Full model       -104.672         4
  Fitted model       -105.397         2       1.44976      2          0.4844
 Reduced model       -106.377         1       3.40987      3          0.3326

          AIC:         214.794

                                Goodness  of  Fit
                                                                      Scaled
    Dose      Est._Prob.    Expected    Observed     Size       Residual
 ----------------------------------------------------------------------------
   0.0000     0.1859         9.296     9.000          50       -0.108
  97.0000     0.1979         9.698     8.000          49       -0.609
 291.0000     0.2209        11.043    14.000          50        1.008
1050.0000     0.2993        14.963    14.000          50       -0.298

 Chi^2 = 1.49      d.f. = 2        P-value = 0.4754

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        721.275
            BMDL =        276.216
```

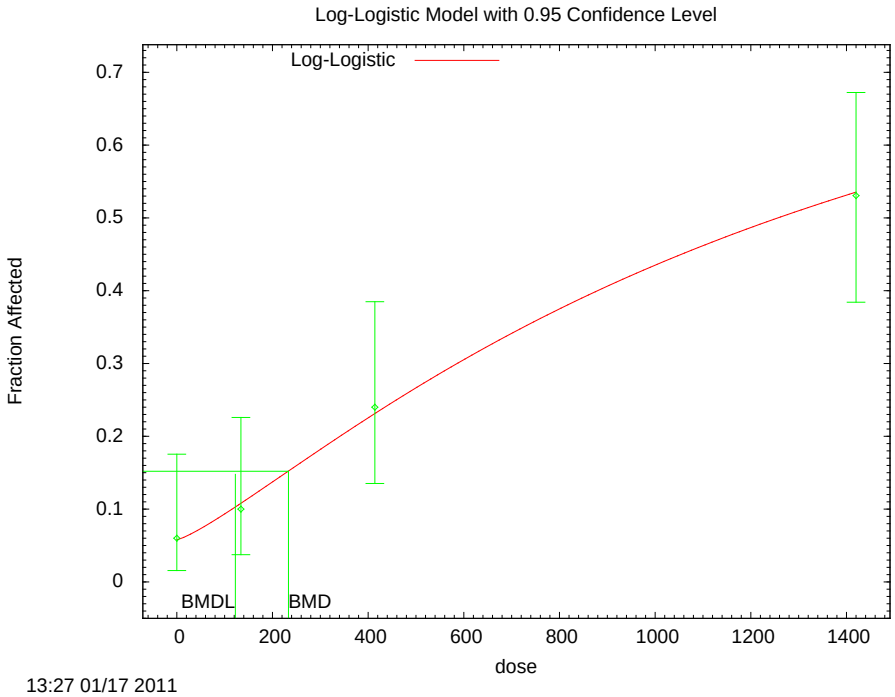
Table C-15. Summary of BMD modeling results for incidence of mineralization in the kidney (inner stripe outer medulla) of female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b]	0.70	-0.27	184.21	116.20	76.96	229.86	158.09
Logistic	0.31	1.22	184.34	257.38	205.80	451.19	369.40
Log-Logistic[b,c]	**0.80**	**-0.18**	**184.12**	**127.12**	**57.98**	**233.39**	**122.40**
Log-Probit[b]	0.53	0.80	183.33	253.31	189.78	364.28	272.92
Multistage (1-degree)[d]	0.92	-0.34	182.23	104.00	76.86	213.63	157.88
Probit	0.38	1.14	183.96	234.00	188.80	417.63	343.46
Weibull[b]	0.69	-0.28	184.22	113.82	76.94	227.40	158.04

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Selected model; the model with the lowest $BMDL_{10}$ was selected because BMDL values for models providing adequate fit differed by more than threefold.
[d]Betas restricted to ≥0.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Umeda et al. (2005).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
          Logistic Model. (Version: 2.13; Date: 10/28/2009)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/minmedulla/female/lnl_minmedullF_loglogistic.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/minmedulla/female/lnl_minmedullF_loglogistic.plt
                                                  Mon Jan 17 13:27:41 2011
 =================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background+(1-background)/[1+EXP(-
intercept-slope*Log(dose))]
Dependent variable = incidence
Independent variable = dose
Slope parameter is restricted as slope >= 1
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =         0.06
                     intercept =      -9.5037
                         slope =      1.31777

           Asymptotic Correlation Matrix of Parameter Estimates
             background    intercept        slope
background            1        -0.48         0.44
intercept         -0.48            1        -0.99
     slope         0.44        -0.99            1

                              Parameter Estimates
                                                    95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background          0.05773               *                   *                   *
      intercept         -8.90345               *                   *                   *
          slope          1.22989               *                   *                   *
* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

```
    Full model        -89.0288         4
  Fitted model        -89.0609         3     0.0641982      1            0.8
 Reduced model        -107.593         1       37.1286      3         <.0001

          AIC:         184.122

                                Goodness  of  Fit
                                                                      Scaled
    Dose      Est._Prob.     Expected     Observed      Size        Residual
  ----------------------------------------------------------------------------
    0.0000     0.0577          2.887      3.000           50         0.069
  134.0000     0.1078          5.391      5.000           50        -0.178
  414.0000     0.2307         11.535     12.000           50         0.156
 1420.0000     0.5344         26.187     26.000           49        -0.053

 Chi^2 = 0.06      d.f. = 1        P-value = 0.8006

Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        233.39
            BMDL =        122.401
```

Table C-16. BMD model results for serum LDH activity in female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{1RD}	$BMDL_{1RD}$
All doses								
Constant variance								
Hill[b]	<0.0001	NA	0.00	1,687.59	CF	CF	182.66	0.0000
Linear[c]	<0.0001	0.38	0.34	1,685.52	2,914.91	1,491.53	465.81	0.0026
Polynomial (2-degree)[c]	<0.0001	0.30	0.34	1,686.01	2,882.07	1,450.54	465.80	0.0011
Polynomial (3-degree)[c]	<0.0001	0.93	0.31	1,683.73	3,194.19	1,595.47	465.86	1.1×10^{-8}
Power[d]	<0.0001	0.93	0.31	1,683.73	3,193.16	1,449.38	465.81	0.0036
Non constant variance								
Hill	0.91	NA	-0.22	1,461.52	72.34	CF	161.83	107.12
Linear[b]	0.91	<0.0001	5.08	1,544.20	-9,999.00	720.55	53.40	19.49
Polynomial (2-degree)[b]	0.91	<0.0001	1.86	1,537.72	554.86	25.81	42.35	6.96
Polynomial (3-degree)[b]	0.91	<0.0001	5.08	1,544.20	-9,999.00	1,947.93	53.40	0.88
Power[d]	0.91	<0.0001	1.33	1,486.07	60.83	41.31	107.91	81.24

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be positive.
[d]Restrict power ≥1.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{1RD}$ = dose associated with a 100% RD from control mean value); CF = computation failed; NA = not applicable (degrees of freedom for the test of mean fit are ≤0, the χ^2 test for fit is not valid)

Source: Umeda et al. (2005).

The constant variance models did not fit the variance data. The nonconstant variance models did not fit the means data. Therefore, none of the models provided an adequate fit to the data on serum LDH activity in female mice exposed to biphenyl in the diet for 2 years.

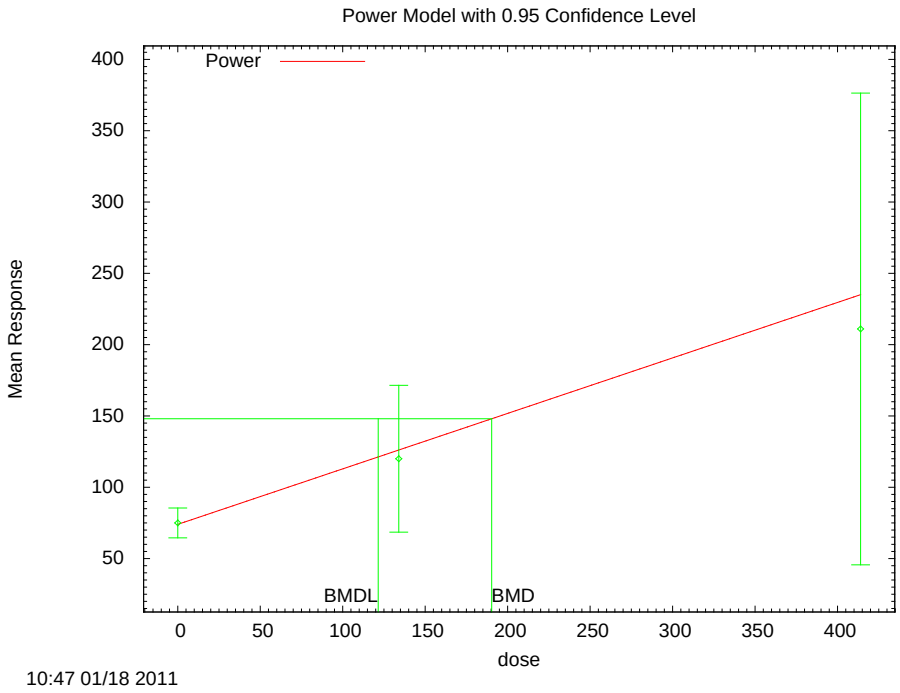
Table C-17. BMD modeling results for serum AST activity in female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{1RD}	$BMDL_{1RD}$
All doses								
Constant variance								
Hill[b]	<0.0001	NA	-5.69×10^7	1,264.30	6,722.40	566.24	213.62	0.00
Linear[c], Polynomial (2-degree)[c], Power[d]	<0.0001	0.72	0.68	1,260.96	1,826.88	1,205.47	595.87	135.74
Non constant variance								
Hill[b]	0.52	NA	0.82	1,121.84	83.86	CF	154.69	114.05
Linear[c]	0.52	<0.0001	5.04	1,219.20	CF	90.71	21.60	2.76
Polynomial (2-degree)[c]	0.52	<0.0001	-2.55×10^9	8.00	0.00	CF	185.08	CF
Power[d]	0.52	<0.0001	-2.13	1,164.51	106.70	69.43	150.64	110.24
Highest dose dropped								
Constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c], Polynomial (2-degree)[c], Power	<0.0001	0.99	0.01	826.48	648.56	372.37	229.54	33.18
Non constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c]	0.78	<0.0001	3.24×10^8	6	0	CF	228.57	CF
Polynomial (2-degree)[c]	0.78	<0.0001	-2.20×10^9	8	0	CF	219.67	CF
Power[d,e]	**0.78**	**0.28**	**-0.29**	**709.33**	**72.36**	**44.29**	**190.33**	**121.53**

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be positive.
[d]Restrict power ≥1.
[e]Selected model; only model providing adequate fit to modeled variance and means.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{1RD}$ = dose associated with a 100% RD from control mean value); CF = computation failed; NA = not applicable (degrees of freedom for the test of mean fit are ≤0, the χ^2 test for fit is not valid)

Source: Umeda et al. (2005).



BMD and BMDL indicated are associated with a twofold increase from control (1RD), and are in units of mg/kg-day.

```
=================================================================
          Power Model. (Version: 2.16;  Date: 10/28/2009)
          Input Data File: C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/AST/pow_ASTFHDD_power.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/AST/pow_ASTFHDD_power.plt
                                                    Tue Jan 18 10:47:11 2011
 =================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is: Y[dose] = control + slope * dose^power
Dependent variable = mean
Independent variable = dose
The power is restricted to be greater than or equal to 1
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
Total number of dose groups = 3
Total number of records with missing values = 0
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
                        lalpha =       10.765
                           rho =            0
                       control =           75
                         slope =     0.369536
                         power =     0.980467

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -power have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
                lalpha          rho      control        slope
    lalpha           1           -1        -0.43         0.85
       rho          -1            1         0.37        -0.89
   control       -0.43         0.37            1        -0.17
     slope        0.85        -0.89        -0.17            1

                              Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        lalpha         -12.9059          4.06805           -20.8791            -4.93268
           rho          4.54893         0.905641             2.7739             6.32395
       control          74.0253          5.21212            63.8097             84.2409
```

```
          slope          0.38893         0.113823            0.165841            0.61202
          power                1              NA
NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
has no standard error.

     Table of Data and Estimated Values of Interest
Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------
    0    28         75           74          27         28.1          0.183
  134    20        120          126         110         94.6          -0.29
  414    22        211          235         373          390         -0.289

      Model Descriptions for likelihoods calculated
Model A1:        Yij = Mu(i) + e(ij)  Var{e(ij)} = Sigma^2
Model A2:        Yij = Mu(i) + e(ij)  Var{e(ij)} = Sigma(i)^2
Model A3:        Yij = Mu(i) + e(ij)  Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user
Model  R:         Yi = Mu + e(i)  Var{e(i)} = Sigma^2

                     Likelihoods of Interest
            Model      Log(likelihood)   # Param's      AIC
              A1         -410.240404            4     828.480807
              A2         -350.033965            6     712.067929
              A3         -350.072753            5     710.145506
          fitted         -350.666161            4     709.332321
               R         -412.701435            2     829.402870

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

                    Tests of Interest
   Test    -2*log(Likelihood Ratio)  Test df        p-value
   Test 1              125.335          4          <.0001
   Test 2              120.413          2          <.0001
   Test 3            0.0775771          1          0.7806
   Test 4              1.18681          1           0.276

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

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

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

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

               Benchmark Dose Computation
Specified effect =          1
Risk Type        =     Relative risk
Confidence level =          0.95
                   BMD = 190.33
                  BMDL = 121.534
```

Table C-18. BMD modeling results for serum ALT activity in female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{1RD}	$BMDL_{1RD}$
All doses								
Constant variance								
Hill[b]	<0.0001	NA	9.61×10^{-7}	1,167.39	3,911.09	436.97	160.82	0.00
Linear[c], Polynomial (2-degree)[c], Power[d]	<0.0001	0.55	0.94	1,164.57	1,613.62	1,106.30	412.90	38.31
Non constant variance								
Hill[b]	0.78	NA	-0.49	1,013.25	116.28	CF	148.75	121.30
Linear[c]	0.78	<0.0001	1.69×10^{10}	6	0	CF	419.08	CF
Polynomial (2-degree)[c]	0.78	<0.0001	-1.39×10^{11}	8	0	CF	87.64	CF
Power[d]	0.78	<0.0001	-1.88	1,047.49	90.73	62.72	108.55	77.76
Highest dose dropped								
Constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c],	<0.0001	0.79	-0.22	756.72	518.80	324.41	116.10	0.00
Polynomial (2-degree)[c]	<0.0001	NA	4.25×10^{-7}	758.65	488.92	325.96	170.36	0.00
Power[d]	<0.0001	NA	-3.00×10^{-9}	758.65	497.95	325.96	167.69	0.00
Non constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c]	0.89	<0.0001	-2.59×10^{9}	6	0	CF	111.13	CF
Polynomial (2-degree)[c]	0.89	<0.0001	-5.85×10^{7}	8	0	CF	169.57	CF
Power[d]	0.89	NA	0.10	631.43	110.52	67.61	172.25	117.98

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be positive.
[d]Restrict power ≥1.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{1RD}$ = dose associated with a 100% RD from control mean value); CF = computation failed; NA = not applicable

Source: Umeda et al. (2005).

The constant variance models did not fit the variance data. The nonconstant variance models fit the variance data, but failed to fit the means data. When the data from the highest dose group were dropped, the constant variance models did not fit the variance data. The nonconstant variance models did not fit the means data. Therefore, none of the models provided an adequate fit to the data on serum ALT activity in female mice exposed to biphenyl in the diet for 2 years.

Table C-19. BMD modeling results for serum AP activity in female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{1RD}	$BMDL_{1RD}$
All doses								
Constant variance								
Hill[b]	<0.0001	NA	-4.74×10^{-8}	1,240.81	642.90	320.63	540.57	180.68
Linear[c], Polynomial (2-degree)[c], Power[d]	<0.0001	0.31	1.32	1,239.14	1,253.51	919.17	1,208.38	720.75
Non constant variance								
Hill[b]	0.006	NA	-0.93	1,180.07	147.47	CF	177.26	CF
Linear[c]	0.006	<0.0001	5.04	1,334.76	-9,999.00	244.46	28.02	0.05
Polynomial (2-degree)[c]	0.006	<0.0001	-2.57×10^{11}	8	0	CF	390.64	CF
Polynomial (3-degree)[c]	0.006	<0.0001	1.89	1,242.58	1,495.81	213.20	1,506.34	333.91
Power[d]	0.006	<0.0001	1.41	1,236.21	665.13	345.69	815.01	482.17
Highest dose dropped								
Constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c],	<0.0001	0.55	-0.51	868.21	617.91	361.78	487.67	201.11
Polynomial (2-degree)[c]	<0.0001	0.95	-0.05	867.85	510.80	393.46	467.69	315.45
Power[d]	<0.0001	NA	1.09E-8	869.84	499.45	372.60	464.35	213.97
Non constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c]	0.77	<0.0001	4.52×10^{9}	6	0	CF	465.02	CF
Polynomial (2-degree)[c]	0.77	NA	0.13	794.19	287.55	183.20	480.63	334.12
Power[d]	0.77	NA	-0.21	794.19	285.46	179.35	482.75	333.04

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be positive.
[d]Restrict power ≥1.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{1RD}$ = dose associated with a 100% RD from control mean value); CF = computation failed; NA = not applicable

Source: Umeda et al. (2005).

The constant variance models did not fit the variance data. The nonconstant variance models fit the variance data, but failed to fit the means data. When the data from the highest dose group were dropped, the constant variance models did not fit the variance data. The nonconstant variance models fit the variance data, but did not fit the means data. Therefore, none of the models provided an adequate fit to the data on serum AP activity in female mice exposed to biphenyl in the diet for 2 years.

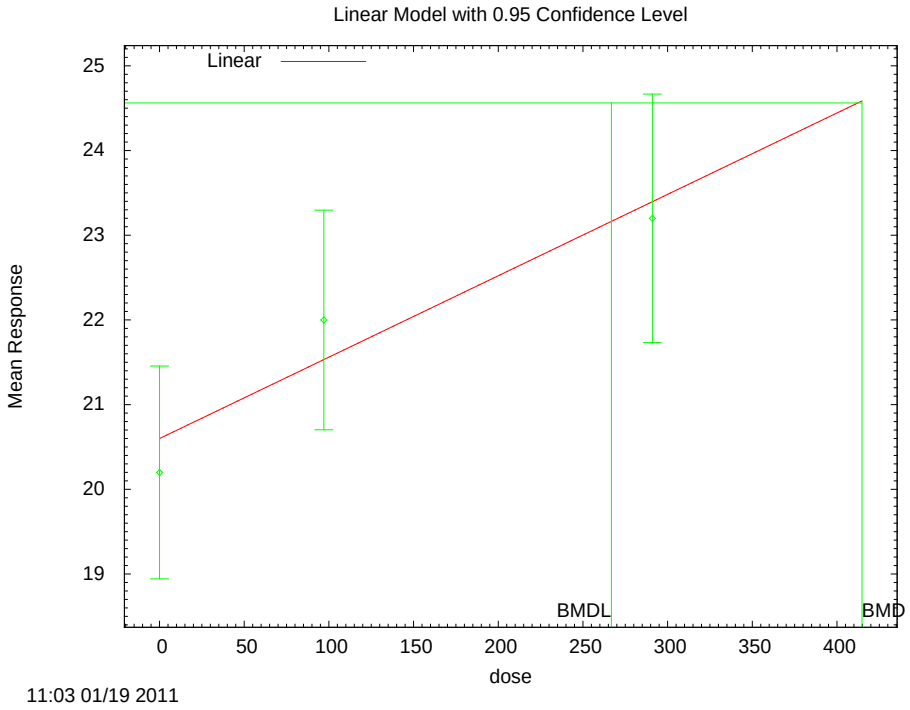
Table C-20. BMD modeling results for changes in BUN levels (mg/dL) in male BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{1RD}	$BMDL_{1RD}$
Males								
All doses								
Constant variance								
Hill[b]	0.03	NA	0.25	540.50	CF	CF	CF	CF
Linear[c,d], Polynomial (2-degree)[c], Power	0.03	0.01	-2.00	545.04	2,254.69	1,288.77	12,777.10	7,154.72
Non constant variance								
Hill[b]	0.01	NA	0.25	542.49	CF	CF	CF	CF
Linear[c]	0.01	0.28	-1.95	540.78	3,134.77	1,690.32	15,745.20	8,512.03
Polynomial (2-degree)[c]	0.01	0.13	-2.23	542.57	2,029.81	1,459.55	4,649.85	3,312.21
Polynomial (3-degree)[c]	0.01	0.13	-2.25	542.52	1,688.06	1,324.21	2,974.25	2,291.81
Power[d]	0.01	0.13	-2.32	542.51	1,170.31	1,092.10	1,334.64	1,196.80
Highest dose dropped								
Constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c], Polynomial (2-degree)[c], Power[d]	**0.49**	**0.32**	**0.77**	**420.23**	**414.78**	**266.77**	**2,140.93**	**1,335.54**

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be positive.
[d]Restrict power ≥1.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{1RD}$ = dose associated with a 100% RD from control mean value); CF = computation failed; NA = not applicable

Source: Umeda et al. (2005).



BMD and BMDL indicated are associated with a 1SD change from control, and are in units of mg/kg-day.

```
================================================================
          Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/BUN/male/lin_BUNMHDD_linear.(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/BUN/male/lin_BUNMHDD_linear.plt
                                                    Wed Jan 19 11:03:37 2011
 ================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
Dependent variable = mean
Independent variable = dose
rho is set to 0
The polynomial coefficients are restricted to be positive
A constant variance model is fit
Total number of dose groups = 3
Total number of records with missing values = 0
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
                          alpha =      16.1929
                            rho =            0   Specified
                         beta_0 =      20.5429
                         beta_1 =   0.00972018

           Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -rho have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
                  alpha       beta_0       beta_1
     alpha            1    -3.8e-008     3.2e-008
    beta_0    -3.8e-008            1        -0.74
    beta_1     3.2e-008        -0.74            1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha          15.8907          2.14271            11.6911             20.0904
        beta_0           20.576         0.566499            19.4657             21.6863
```

```
         beta_1        0.0096108       0.00317579          0.00338636           0.0158352

     Table of Data and Estimated Values of Interest
Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    34       20.2         20.6          3.6         3.99          -0.55
   97    39         22         21.5            4         3.99           0.77
  291    37       23.2         23.4          4.4         3.99         -0.264

 Model Descriptions for likelihoods calculated
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2
Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that were specified by the user
Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2

                     Likelihoods of Interest
            Model      Log(likelihood)   # Param's      AIC
             A1         -206.630664            4     421.261329
             A2         -205.915695            6     423.831391
             A3         -206.630664            4     421.261329
          fitted        -207.115525            3     420.231050
              R         -211.514015            2     427.028031

                   Explanation of Tests

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

                     Tests of Interest
   Test    -2*log(Likelihood Ratio)  Test df        p-value
   Test 1              11.1966          4         0.02444
   Test 2              1.42994          2          0.4892
   Test 3              1.42994          2          0.4892
   Test 4             0.969721          1          0.3247

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

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

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

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

Benchmark Dose Computation
Specified effect =             1
Risk Type        =     Estimated standard deviations from the control mean
Confidence level =          0.95
             BMD =        414.775
            BMDL =        266.77
```

Table C-21. BMD modeling results for changes in BUN levels (mg/dL) in female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	BMD_{1RD}	$BMDL_{1RD}$
All doses								
Constant variance								
Hill[b]	<0.0001	NA	-3.45×10^{-8}	603.61	CF	CF	CF	CF
Linear[c], Polynomial (2-degree)[c], Power[d]	<0.0001	0.38	1.18	601.53	1,869.01	1,224.15	2,507.85	1,434.76
Non constant variance								
Hill[b]	0.08	NA	-1.21	493.48	141.72	CF	CF	CF
Linear[c], Polynomial (2-degree)[c], Power[d]	0.08	<0.0001	-1.63	590.70	519.60	216.41	1,191.69	683.73
Highest dose dropped								
Constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c],	<0.0001	0.50	-0.57	417.59	744.99	403.07	921.79	410.67
Polynomial (2-degree)[c]	<0.0001	0.82	-0.18	417.19	555.48	413.38	627.58	432.73
Power[d]	<0.0001	NA	-2.11×10^{-10}	419.13	430.03	414.77	436.97	417.75
Non constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c]	0.23	0.07	-1.38	300.36	180.70	114.17	1,416.07	916.09
Polynomial (2-degree)[c]	0.23	NA	-0.93	299.05	263.22	152.60	842.06	495.16
Power[d]	0.23	<0.0001	-0.93	297.05	256.90	151.17	925.84	490.39

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be positive.
[d]Restrict power ≥1.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{1RD}$ = dose associated with a 100% RD from control mean value); CF = computation failed; NA = not applicable

Source: Umeda et al. (2005).

The constant variance models did not fit the variance data. The nonconstant variance models fit the variance data, but failed to fit the means data. When the data from the highest dose group were dropped, the constant variance models did not fit the variance data. The nonconstant variance models fit the variance data, but did not fit the means data. Therefore, none of the models provided an adequate fit to the data on BUN levels in female mice exposed to biphenyl in the diet for 2 years.

Table C-22. BMD modeling results for changes in mean terminal body weight in male BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	$BMD_{0.1RD}$	$BMDL_{0.1RD}$
All doses								
Constant variance								
Hill[b]	<0.0001	0.03	-1.68	716.95	459.61	390.85	358.30	316.09
Linear[c], Power[d]	<0.0001	0.10	-1.68	714.95	460.46	391.75	359.04	316.87
Polynomial (3-degree)[c]	<0.0001	0.03	-1.66	716.89	498.04	392.48	390.52	317.33
Non constant variance								
Hill[b]	0.002	NA	-1.52	704.84	600.48	CF	421.46	325.00
Linear[c],	0.002	0.59	-1.52	701.13	541.68	460.24	357.54	326.02
Polynomial (3-degree)[c]	0.002	0.44	-1.42	702.64	643.20	467.09	450.96	328.74
Power[d]	0.002	0.38	-1.51	702.84	600.89	464.26	421.53	327.62
Highest dose dropped								
Constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c], Polynomial (2-degree)[c], Power[d]	0.01	0.05	-1.49	560.11	566.99	328.79	400.33	238.24
Non constant variance								
Hill[b]	Not modeled; number of dose groups less than number of model parameters							
Linear[c], Polynomial (2-degree)[c], Power[d]	0.18	0.001	-1.5	562.10	561.56	308.43	398.66	235.32

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be negative.
[d]Restrict power ≥1.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{0.1RD}$ = dose associated with a 10% RD from control mean value); CF = computation failed; NA = not applicable

Source: Umeda et al. (2005).

The constant variance models did not fit either the variance data or the means data. The nonconstant variance models failed to fit the variance data. When the data from the highest dose group were dropped, the constant variance models did not fit either the variance data or the means data. The nonconstant variance models did not fit the means data. Therefore, none of the models provided an adequate fit to the data on mean terminal body weight in male mice exposed to biphenyl in the diet for 2 years.

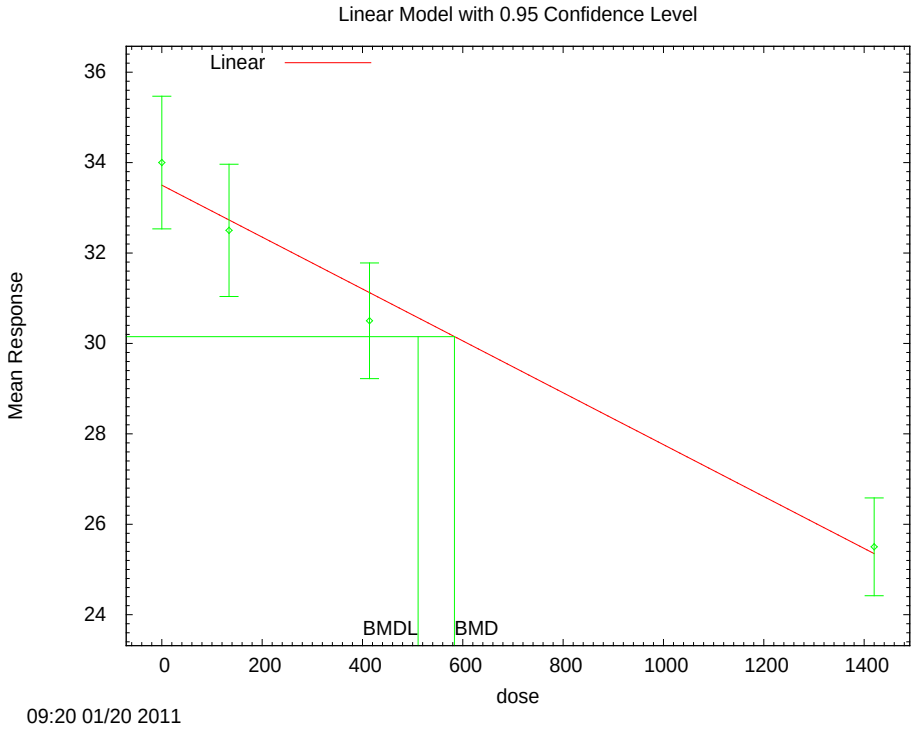
Table C-23. BMD modeling results for changes in mean terminal body weight in female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Largest residual	AIC	BMD_{1SD}	$BMDL_{1SD}$	$BMD_{0.1RD}$	$BMDL_{0.1RD}$
All doses								
Constant variance								
Hill[b]	0.36	0.80	-0.21	382.59	387.90	230.17	397.06	243.57
Linear[c,d], Polynomial (2-degree)[c], Power[e]	**0.36**	**0.42**	**-0.93**	**382.26**	**584.12**	**489.94**	**583.33**	**510.85**

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Restrict n > 1.
[c]Coefficients restricted to be negative.
[d]Selected model; the model with the lowest AIC was selected because BMDL values for models providing adequate fit did not differ by more than threefold.
[e]Restrict power ≥1.

BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{1SD}$ = dose associated with 1 SD from control mean value; $_{0.1RD}$ = dose associated with a 10% RD from control mean value); CF = computation failed; NA = not applicable

Source: Umeda et al. (2005).



BMD and BMDL indicated are associated with a 10% decrease from control (0.1 RD), and are in units of mg/kg-day.

```
================================================================
         Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
         Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/termbdwt/female/lin_termbdwtF_linear.(d)
         Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/termbdwt/female/lin_termbdwtF_linear.plt
                                                  Thu Jan 20 09:20:01 2011
 ================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
Dependent variable = mean
Independent variable = dose
rho is set to 0
The polynomial coefficients are restricted to be negative
A constant variance model is fit
Total number of dose groups = 4
Total number of records with missing values = 0
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
                        alpha =      11.4937
                          rho =            0   Specified
                       beta_0 =      33.4391
                       beta_1 =  -0.00571961

          Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -rho  have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
                  alpha       beta_0       beta_1
     alpha            1    -9.6e-009     9.1e-009
    beta_0    -9.6e-009            1        -0.67
    beta_1     9.1e-009        -0.67            1

                               Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha          11.2518           1.5172             8.27818             14.2255
         beta_0          33.4983         0.432523             32.6505              34.346
         beta_1      -0.00574262      0.000545303          -0.0068114         -0.00467385

     Table of Data and Estimated Values of Interest
Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------
    0    31         34         33.5           4         3.35          0.833
  134    22       32.5         32.7         3.3         3.35          -0.32
  414    25       30.5         31.1         3.1         3.35         -0.925
 1420    32       25.5         25.3           3         3.35          0.264

 Model Descriptions for likelihoods calculated
Model A1:        Yij = Mu(i) + e(ij)  Var{e(ij)} = Sigma^2
Model A2:        Yij = Mu(i) + e(ij)  Var{e(ij)} = Sigma(i)^2
Model A3:        Yij = Mu(i) + e(ij)  Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that were specified by the user
Model  R:         Yi = Mu + e(i)  Var{e(i)} = Sigma^2

                     Likelihoods of Interest
            Model      Log(likelihood)   # Param's      AIC
              A1         -187.261579           5     384.523158
              A2         -185.643849           8     387.287698
              A3         -187.261579           5     384.523158
          fitted         -188.129218           3     382.258435
               R         -226.477701           2     456.955401

                   Explanation of Tests

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

                    Tests of Interest
```

```
   Test    -2*log(Likelihood Ratio)  Test df        p-value
   Test 1              81.6677          6          <.0001
   Test 2              3.23546          3          0.3567
   Test 3              3.23546          3          0.3567
   Test 4              1.73528          2          0.4199

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

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

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

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

             Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =     Relative risk
Confidence level =          0.95
             BMD =        583.327
            BMDL =        510.848
```

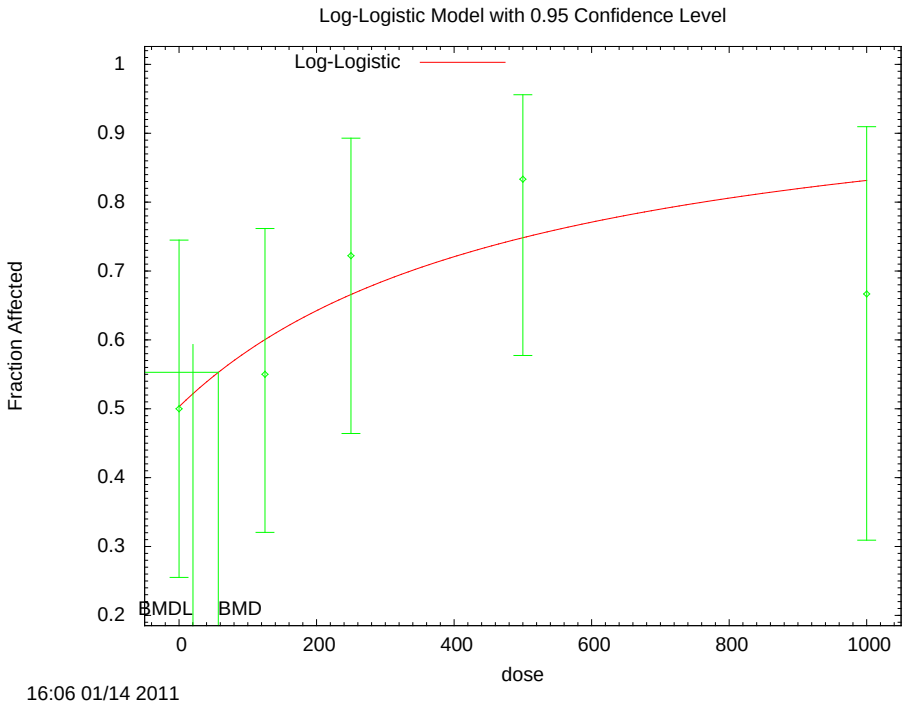
Table C-24. Summary of BMD modeling results for incidence of litters with fetal skeletal anomalies from Wistar rat dams administered biphenyl by gavage on GDs 6–15

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
Gamma[b], Weibull[b], Multistage (1-degree)[c]	0.31	-1.25	106.11	54.45	24.15	111.84	49.61
Logistic	0.28	1.17	106.42	73.97	36.73	149.18	73.79
Log-Logistic[b,d]	**0.41**	**-1.32**	**105.33**	**27.03**	**9.59**	**57.06**	**20.24**
Log-Probit[b]	0.23	-1.59	106.55	125.14	55.10	179.97	79.23
Probit	0.28	1.20	106.50	79.59	41.02	160.27	82.37

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0.
[d]Selected model; the model with the lowest BMDL was selected because BMDL values for models providing adequate fit differed by more than threefold; this model also had the lowest AIC.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk; $_5$ = dose associated with 5% extra risk)

Source: Khera et al. (1979).



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
================================================================
         Logistic Model. (Version: 2.13; Date: 10/28/2009)
         Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/develop/anomlitt/lnl_anomlitt_loglogistic.(d)
         Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/rat/develop/anomlitt/lnl_anomlitt_loglogistic.plt
                                                  Fri Jan 14 16:06:43 2011
 ================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background+(1-background)/[1+EXP(-
intercept-slope*Log(dose))]
Dependent variable = incidence
Independent variable = dose
Slope parameter is restricted as slope >= 1
Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =          0.5
                     intercept =     -6.54827
                         slope =            1

          Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -slope have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
             background    intercept
background            1        -0.77
intercept         -0.77            1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.503241            *                *                   *
      intercept         -6.24131            *                *                   *
```

```
         slope               1           *               *                  *
* - Indicates that this value is not calculated.

                      Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -49.327         5
   Fitted model        -50.6629         2       2.67182      3           0.445
  Reduced model        -52.2232         1       5.79233      4          0.2152

          AIC:         105.326

                                Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.5032         8.052     8.000          16       -0.026
  125.0000     0.6005        12.010    11.000          20       -0.461
  250.0000     0.6659        11.986    13.000          18        0.507
  500.0000     0.7483        13.469    15.000          18        0.831
 1000.0000     0.8315         7.483     6.000           9       -1.321

 Chi^2 = 2.90      d.f. = 3        P-value = 0.4065

   Benchmark Dose Computation
Specified effect =         0.1
Risk Type        =      Extra risk
Confidence level =         0.95
             BMD =        57.0591
            BMDL =        20.2399
```

APPENDIX D. BENCHMARK MODELING FOR THE ORAL SLOPE FACTOR

The mouse liver tumor dataset from Umeda et al. (2005) for which dose-response modeling was performed is shown in Table D-1.

Table D-1. Incidences of liver adenomas or carcinomas (combined) in female BDF_1 mice fed diets containing biphenyl for 2 years

Biphenyl dietary concentration (ppm)	0	667	2,000	6,000
Reported dose (mg/kg-d)	0	134	414	1,420
HED (mg/kg-d)	0	19	59	195
Tumor incidence				
Adenoma or carcinoma (combined)	3/48[a]	8/50	16/49[a,b]	14/48[a,c]

[a]Two control, one mid-dose, and two high-dose female mice were excluded from denominators because they died prior to week 52. It is assumed that they did not have tumors and were not exposed for a sufficient time to be at risk for developing a tumor. Umeda et al. (2005) did not specify the time of appearance of the first tumor.
[b]Significantly different from controls ($p < 0.05$) according to Fisher's exact test.
[c]Significantly different from controls ($p < 0.01$) according to Fisher's exact test.

Source: Umeda et al. (2005).

Summaries of the BMDs, BMDLs, and the derived oral slope factors for the modeled mouse data are presented in Table D-2, followed by the plot and model output file from the best-fitting model. The animals in the highest dose group, while exhibiting a statistically significantly increased incidence in liver tumors compared with controls, did not show a monotonic increase in tumor response compared with the responses at the lower doses. To better estimate responses in the low-dose region, the high-dose group was excluded as a means of improving the fit of the model in the region of interest.

Table D-2. Model predictions for liver tumors (adenomas or carcinomas combined) in female BDF_1 mice exposed to biphenyl in the diet for 2 years

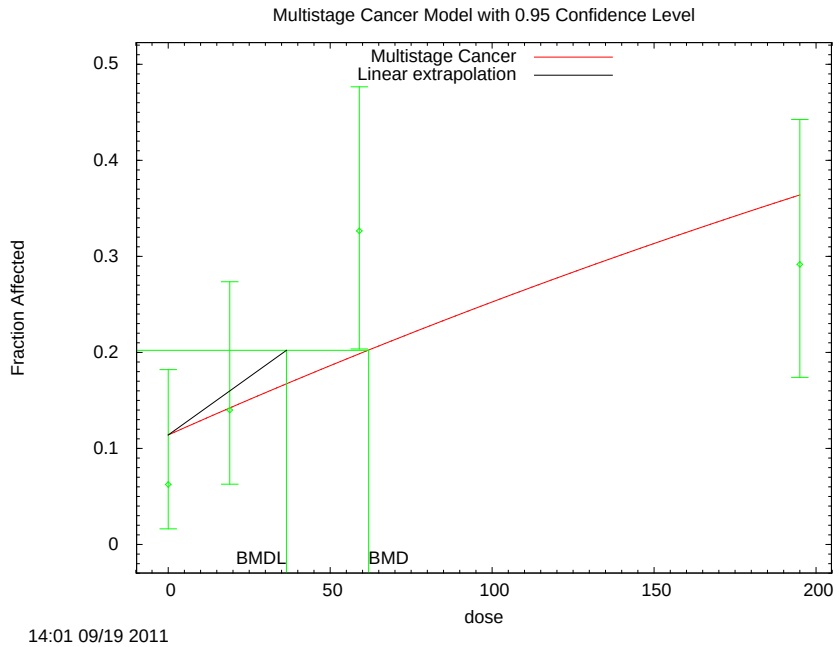
Model	Goodness of fit			Benchmark result (mg/kg-d)		
	χ^2 *p*-value[a]	Largest residual	AIC	BMD_{HED10}	$BMDL_{HED10}$	Cancer slope factor (risk per mg/kg-d)
All doses						
Multistage (1-, 2-, 3-degree)[b], Gamma[c], Weibull[c]	0.03	2.14	197.37	64.76	37.29	0.003
Logistic	0.01	2.31	198.96	104.91	71.27	0.001
Log-Logistic[c]	0.04	1.97	196.62	50.68	26.80	0.004
Log-Probit[c]	0.005	2.58	201.06	128.52	74.43	0.001
Probit	0.01	2.30	198.80	100.16	67.23	0.001
Highest dose dropped						
Multistage (1-degree)[b,d]	**0.96**	**0.04**	**132.32**	**18.72**	**12.15**	**0.008**
Multistage (2-degree)[b]	0.96	0.04	132.32	18.72	12.15	0.008

[a]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[b]Betas restricted to ≥0.
[c]Power restricted to ≥1.
[d]Selected model.

BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{HED10}$ = HED associated with 10% extra risk

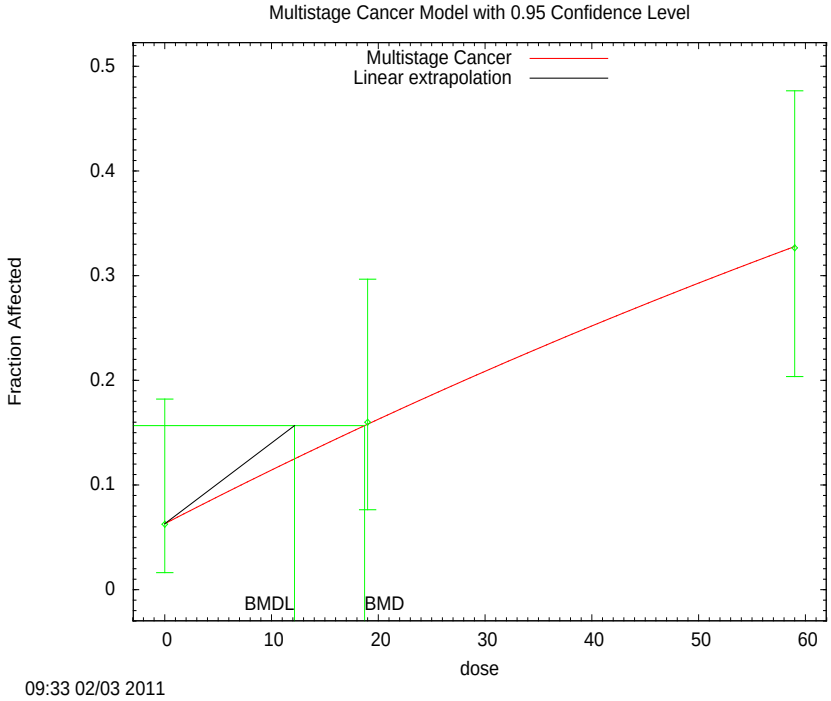
Source: Umeda et al. (2005).

BMDS graph of multistage (1-degree) model that includes data from the highest dose group:



The BMDS graph of multistage (1-degree) model that includes data from the highest dose group. BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

BMDS graph of multistage (1-degree) model with the highest dose group dropped:



BMD and BMDL indicated are associated with an extra risk of 10%, and are in units of mg/kg-day.

```
=================================================================
          Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
          Input Data File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/livertumor/female/revised_n/msc_livtumFrev2HDD_MS_1.
(d)
          Gnuplot Plotting File:
C:/Storage/USEPA/IRIS/biphenyl/2011/BMD/mice/livertumor/female/revised_n/msc_livtumFrev2HDD_MS_1.
plt
                                                    Thu Feb 03 09:33:34 2011
=================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008
****  We are sorry but Relative Function and Parameter Convergence are currently unavailable in
this model.  Please keep checking the web site for model updates which will eventually
incorporate these convergence criterion.  Default values used.  ****

                 Default Initial Parameter Values
                    Background =    0.0638384
                       Beta(1) =   0.00559363

           Asymptotic Correlation Matrix of Parameter Estimates
             Background      Beta(1)
Background            1         -0.7
   Beta(1)         -0.7            1

                                Parameter Estimates
                                                    95.0% Wald Confidence Interval
```

```
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background        0.0630397            *                *                   *
       Beta(1)       0.00562948            *                *                   *
* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -64.1585         3
   Fitted model       -64.1595         2     0.0019921      1          0.9644
  Reduced model        -70.107         1       11.8969      2         0.00261

          AIC:         132.319

                                  Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
   0.0000     0.0630         3.026     3.000          48       -0.015
  19.0000     0.1581         7.904     8.000          50        0.037
  59.0000     0.3278        16.064    16.000          49       -0.019

 Chi^2 = 0.00      d.f. = 1        P-value = 0.9644

Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        18.7158
            BMDL =        12.1518
            BMDU =        36.3895
Taken together, (12.1518, 36.3895) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor =    0.00822924
```