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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)

August 2013

U.S. Environmental Protection Agency
Washington, DC

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CONTENTS—TOXICOLOGICAL REVIEW OF BIPHENYL (CAS No. 92-52-4)

LIST OF TABLES

LIST OF FIGURES

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
CASRN	Chemical Abstracts Service Registry Number
CCRIS	Chemical Carcinogenesis Research Information System
COMT	catechol-O-methyltransferase
CVSF	conduction velocity of the slowest motor fibers
CYP	cytochrome P-450
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
EEG	electroencephalography
EHEN	N-ethyl-N-hydroxyethylnitrosamine
EMG	electromyographic
ENMG	electroneuromyography
GC	gas chromatography
GD	gestation day
GLP	Good Laboratory Practice
GOT	glutamate oxaloacetate transaminase
GPT	glutamate pyruvate transaminase
HED	human equivalent doses
HERO	Health and Environmental Research Online
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substances Data Bank
i.p.	intraperitoneal or intraperitoneally
IRIS	Integrated Risk Information System
JBRC	Japan Bioassay Research Center
K_{ow}	octanol/water partition coefficient
K_m	Michaelis constant
K_p	permeability coefficient
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
MCV	motor conduction velocity
MS	mass spectrometry
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level

NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCO	palmitoyl CoA oxidase
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
RTECS	Registry of Toxic Effects of Chemical Substances
SCE	sister chromatid exchange
SD	standard deviation
SULT	sulphotransferase
TLV	threshold limit value
TMS	trimethylsilyl
TSCATS	Toxic Substances Control Act Test Submissions
TWA	time-weighted average
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), *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), *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011), and *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012).

This Toxicological Review is based on a review and evaluation of the primary, peer-reviewed literature pertaining to biphenyl. The search strategy used to identify this literature, including databases and keywords, and the results of the literature search are described in Appendix B. References from health assessments developed by other national and international health agencies were also examined. Other peer-reviewed information, including review articles, literature necessary for the interpretation of biphenyl-induced health effects, and independent analyses of the health effects data were retrieved and included in the assessment where appropriate. The U.S. Environmental Protection Agency (U.S. EPA) requested public submissions of additional information on biphenyl in December 2007 (U.S. EPA, 2007); no submissions in response to the data call-in were received. A comprehensive literature search was last conducted in September 2012. No major epidemiology studies or subchronic and chronic animal studies on biphenyl were identified since the draft Toxicological Review (dated September 2011) was released for external peer review and public comment.

On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law[1]. The report language included direction to EPA for the Integrated Risk Information System (IRIS) Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde[2]. The report language included the following:

> The Agency shall incorporate, as appropriate, based on chemical-specific data sets and biological effects, the recommendations of Chapter 7 of the National Research Council's Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde into the IRIS process…For draft assessments released in fiscal year 2012, the Agency shall include documentation describing how the Chapter 7 recommendations of the National Academy of Sciences (NAS)

[1]Pub. L. No. 112-74, Consolidated Appropriations Act, 2012.
[2]NRC (2011) Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde.

have been implemented or addressed, including an explanation for why certain recommendations were not incorporated.

The NRC's recommendations, provided in Chapter 7 of the review report, offered suggestions to EPA for improving the development of IRIS assessments. Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in the tables in Appendix F. Where necessary, the documentation includes an explanation for why certain recommendations were not incorporated.

The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that, "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

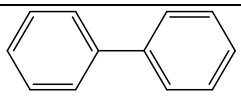
The IRIS biphenyl assessment is in Phase 1 of implementation, which focuses on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focuses on assessments near the end of the development process and close to final posting. Chemical assessments in Phase 2 of the implementation will address all of the short-term recommendations from Appendix F, Table F-1. The IRIS Program is implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Chemical assessments in Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined in Appendix F, Table F-2, including the development of a standardized approach to describe the strength of the evidence for noncancer effects. On May 16, 2012, EPA announced[3] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

[3]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris).

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
Chemical Abstracts Service Registry Number (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. The current major uses of biphenyl are as chemical synthesis intermediates (among them, the sodium salt of 2-hydroxybiphenyl, 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). 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).

Biphenyl 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. 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

Summary. Animal studies indicate that biphenyl is rapidly and readily absorbed following oral exposure. An in vitro study suggests that biphenyl can also be absorbed via dermal exposure. Absorbed biphenyl is not preferentially stored in tissues and is rapidly excreted, principally through the urine. Phase I metabolism by cytochrome P-450 (CYP) enzymes, including CYP1A2 and CYP3A4, in the liver converts biphenyl to a range of hydroxylated metabolites, with 4-dihydroxybiphenyl, 4,4'-dihydroxybiphenyl, and 3,4-dihydroxybiphenyl being the major metabolites. Phase II metabolism catalyzing the conjugation of hydroxylated biphenyl metabolites to sulphate or glucuronic acid occurs mostly in the liver, followed by the intestine and kidney. Absorbed biphenyl is rapidly eliminated from the body, principally as conjugated hydroxylated metabolites in the urine. The toxicokinetic properties of biphenyl are described in more detail in the remainder of this section.

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., 1976b; Meyer et al., 1976a). Results from a study with rats administered radiolabeled biphenyl indicate extensive oral absorption (Meyer et al., 1976a) (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., 1976b).

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., 1976a). 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.

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., 1976a). 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.

Dermal absorption by human skin was measured in an in vitro static diffusion cell model (DuPont, 2005). Epidermis (~0.64 cm^2) was mounted onto an in vitro static diffusion cell, stratum corneum uppermost. An infinite dose (100 µL/cm^2 for a permeability experiment, 20 µL/cm^2 for an exposure rate experiment) of biphenyl in isopropyl myristate vehicle was applied to the epidermal surface, via the donor chamber. Fluid in the receptor chamber was analyzed after different time periods. The study reported a permeability coefficient (K_p) of 6.12×10^{-5} cm/hour, and short-term exposure rates of 258.3 µg equivalents/cm^2/hour (10-minute exposure) and 59.1 µg equivalents/cm^2/hour (60-minute exposure).

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

3.2. DISTRIBUTION

No information was located regarding distribution of absorbed biphenyl in humans, and limited animal data are available. Meyer et al. (1976b) 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., 1976b; Meyer et al., 1976a). 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., 1976b; Meyer et al., 1976a). 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., 1976a) 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	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 reported as a percent of the administered dose.

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.

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, respectively.

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 4-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 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. (1976) and Wiebkin et al. (1984) 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

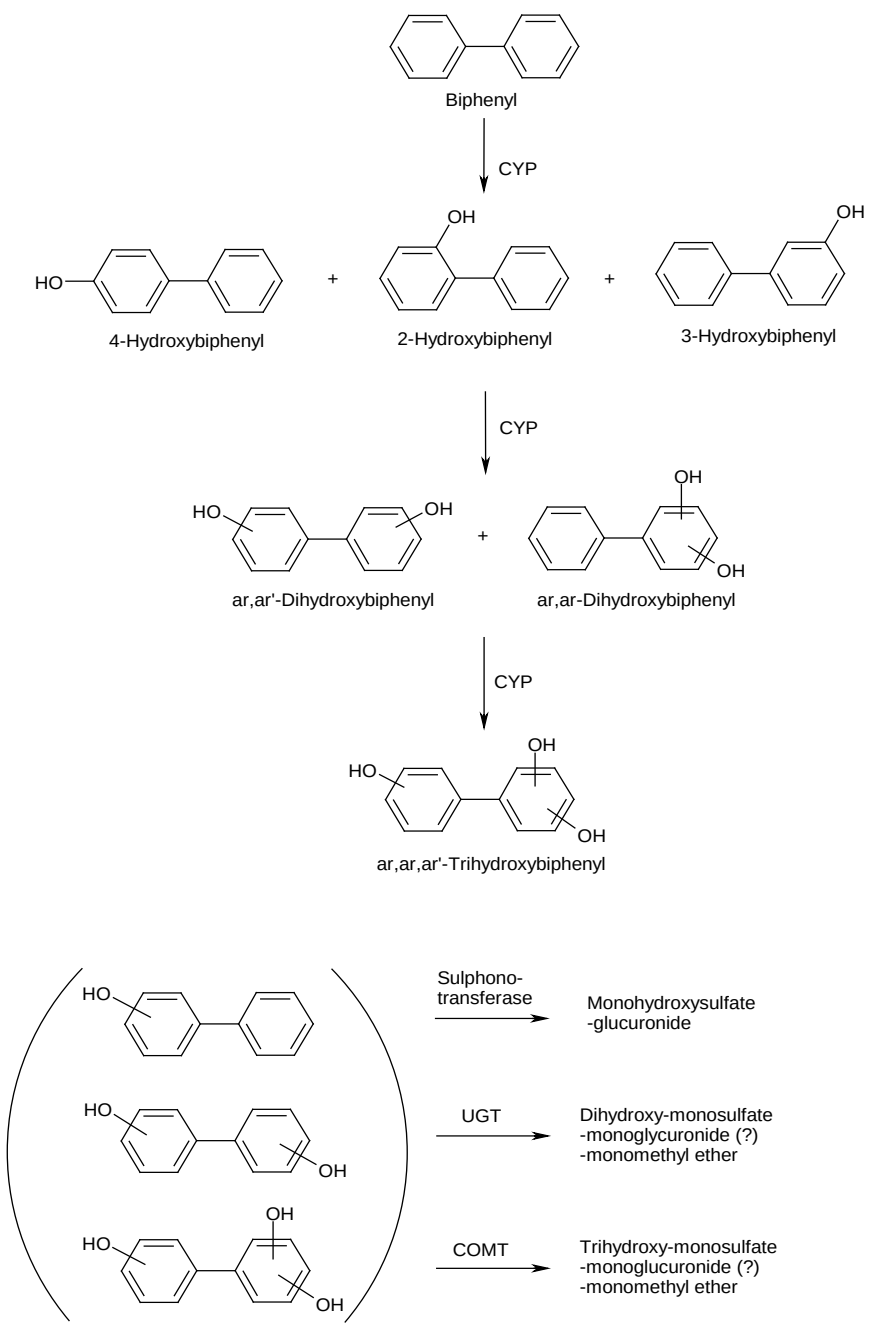
conjugate formation rate in the in vitro experiment. Bianco et al. (1979) reported that rat hepatic microsomes metabolize biphenyl to 4-, 2-, and 3-hydroxybiphenyl, which are conjugated to form glucuronides and sulphates. The 4-hydroxybiphenyl isomer was the major metabolite. The formation of 4-hydroxybiphenyl 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 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 et al. (1976a) 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 (1976).

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 with a nonlinear dose-response relationship (i.e., incidence of bladder tumors of 96% at 1.25% in diet, but no tumors at concentrations ≤0.625%) (Kwok et al., 1999; Hiraga and Fujii, 1984). 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 and Scheline, 1976; Meyer et al., 1976a) (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 and Sites of Metabolism

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 100–500-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., 1976a). 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., 1976b). Although chemical identity analysis of fecal radioactivity was not conducted by Meyer et al. (1976b), 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., 1976a) following oral administration of 100 mg/kg doses of unlabeled biphenyl. In 24-hour urine samples, unchanged biphenyl was not detected; 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., 1976a).

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

Summary. Human studies of biphenyl include assessments of workers exposed to biphenyl during production of biphenyl-impregnated fruit wrapping paper at one mill in Finland and another mill in Sweden. The study of Finish mill workers provided evidence of abnormal electroencephalography (EEG), nerve conduction velocity, and electromyographic (EMG) test results in workers exposed to biphenyl at levels in excess of occupational exposure limits (Seppalainen and Hakkinen, 1975; Häkkinen et al., 1973). Similar neurological findings were not reported in the study of Swedish mill workers whose exposures were likely to have exceeded the occupational exposure limit (Wastensson et al., 2006); however, an increased relative risk of Parkinson's disease was reported.

A case report of a 46-year-old female who worked at a fruit-packing facility in Italy 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, 2001a) 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 engaged in the biphenyl-impregnation process and two other workers exposed to biphenyl elsewhere in the facility 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. Twenty-two of the 33 workers (including the 8 who were hospitalized for testing) were subjected to neurophysiological examinations, including EEG and electroneuromyography (ENMG, consisting of nerve conduction velocity and EMG tests). Fifteen of these 22 workers displayed abnormal findings and 4 displayed borderline findings on one or both of theses tests. 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. Seppalainen and Hakkinen (1975) reported more detailed information about these examinations, and included results for two additional workers for a total of 24, as summarized below.

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 had significantly ($p < 0.001$) slower mean conduction velocity of the slowest motor fibers (CVSF) of the ulnar nerves. Results at the 1-year followup 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)
Median		
MCV	57.7 ± 6.3	58.0 ± 3.8
Ulnar		
MCV	56.3 ± 4.6	56.6 ± 4.0
CVSF	41.4 ± 5.2*	45.5 ± 3.2
Deep peroneal		
MCV	50.2 ± 5.4	50.3 ± 3.5
CVSF	37.7 ± 3.9	38.2 ± 5.6
Posterior tibial		
MCV	43.4 ± 3.9	42.4 ± 4.7

*Statistically significant (*t*-test, $p < 0.05$) as reported by study authors.

SD = standard deviation

Source: Seppalainen and Hakkinen (1975).

Seppalainen and Hakkinen (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. Anomalies in nerve conduction, EEG, and ENMG signals, while small, were consistent with the persistence of incapacity and the incidence of subjective symptoms.

Another study examined the prevalence and incidence of Parkinson's disease among workers at a facility manufacturing biphenyl-impregnated paper in Sweden (Wastensson et al., 2006). The study was prompted by the recognition that three cases seen at a neurological clinic shared a history of work at this workplace. The investigators used company and union records to identify 506 people who had worked in this production process between 1954 and 1970. Vital status was traced through the Swedish National Population registry; 222 had died and 284 were still alive in Sweden in August 2002. The files were missing data for 4 years (1965–1968), and the investigators estimated that this resulted in approximately 30 missing individuals from the at risk pool. Prevalent cases were identified among those still alive through review of medical records as well as a second examination by a study neurologist. Case definition was based on the presence of at least two signs (tremor, rigidity, hypokinesia) and positive response to levodopa (a treatment for Parkinson's disease). The National Hospital Discharge Register, Cause of Death Register, and medical records were examined to determine presence of Parkinson's disease among those who had died. Comparison rates for prevalence of Parkinson's disease was based on age- and sex-specific prevalence rates from a study in eastern Sweden; prevalence risk ratios were calculated for ages <80 years because of the larger variation seen among studies in rates at older ages. The data from the deceased group was not included in these calculations, but were included in analyses of lifetime risk, with comparison rates based on age- and sex-specific data from a study in Olmsted County, Minnesota (a population served by the Mayo Clinic).

Wastensson et al. (2006) identified 5 prevalent cases among the 255 workers ages <80 years compared with 0.9 cases expected, for a relative risk (RR) of 5.6 (95% confidence interval 1.9–13). The mean age at onset of symptoms was 51 years (range 45–55), considerably lower than the mean of 66 years seen in the comparison population. Nine cases were identified among the 222 deceased workers, compared to 4.3 expected (RR 2.1, 95% confidence interval 0.96, 4.0). The clinical features and exposure data for the five living subjects, all of whom were diagnosed with Parkinson's disease 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.

Table 4-3. Exposure data and clinical features for five Parkinson's disease patients with occupational exposure to biphenyl

	Case				
	1	**2**	**3**	**4**	**5**
Exposure data					
Age	63	63	58	54	63
Workplace	Paper Mill 3	Paper Mill 3	Paper Mill 4	Paper Mill 3	Paper Mill 3
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.

Source: Wastensson et al. (2006)

Four of the five prevalent cases 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 that the level of biphenyl in air would likely be greater (more than 2 times higher) 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.

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

Summary. Available oral data for biphenyl include two well-designed 2-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 only at the highest tested dietary concentration, 4,500 ppm; neither the neoplasia nor the calculi were found at lower exposure levels of 1,500 or 500 ppm. An increased incidence of liver tumors (hepatocellular adenomas and carcinomas) was observed in female, but not male, BDF_1 mice exposed to biphenyl at dietary concentrations of 2,000 or 6,000 ppm (Umeda et al., 2005). The database for biphenyl includes studies in rats and mice that did not show clear evidence of carcinogenicity (Shiraiwa et al., 1989; Imai et al., 1983; NCI, 1968;

Ambrose et al., 1960; Dow Chemical Co, 1953), but that were also limited in large part in design, conduct, or reporting of results and were therefore considered less informative for evaluating the carcinogenicity of biphenyl than the studies by Umeda et al. (2005) and Umeda et al. (2002).

Nonneoplastic kidney lesions were reported in 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 incidences of noncancer effects on the kidney (e.g., 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).

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 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 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, 1953). Based on U.S. EPA (1988) subchronic reference values for body weight and food consumption in female Long-Evans rats, these dietary levels corresponded to doses of 10, 30, and 100 mg/kg-day, respectively. 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).

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, based on measurements in 4 rats/group). The biological significance of these decreases in BUN is unclear.

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.

(2004a). Based on U.S. EPA (1988) subchronic reference values for body weight and food consumption (average values for combined sexes), these dietary concentrations corresponded to doses of 93, 374, 747, 1,495, 1,868, and 2,989 mg/kg-day, respectively[4]. 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.

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 decreased by more than 10% compared to 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. (2004b) 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.

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.

[4]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.

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. Hematological parameters and BUN were within normal limits in all groups of animals, and no dose-related effects on final body weight or weights of the lung, kidney, heart, or spleens were observed. 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.

4.2.1.2. *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) conducted by the Japan Bioassay Research Center (JBRC), biphenyl was administered in the diet for 2 years at concentrations of 0, 500, 1,500, or 4,500 ppm (Umeda et al., 2002). Based on time-weighted average (TWA) body weights estimated from the graphically-depicted data (Umeda et al., 2002; Figure 1) and chronic reference values for food consumption in F344 rats (U.S. EPA, 1988), these dietary concentrations corresponded to doses of 36.4, 110, and 378 mg/kg-day, respectively, for males and 42.7, 128, and 438 mg/kg-day, respectively, for females. All animals were examined daily for clinical signs; body weights and food intake were determined once/week for the first 14 weeks and every 4 weeks thereafter. Urinalysis was performed on all surviving rats at week 105. Upon necropsy, all major organs were weighed and tissue samples were subjected to histopathologic examination.

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 statistically 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 reported not to differ statistically significantly 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 (blood in the urine) 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

considered as primary 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 statistically significantly increased urinary pH in the 31 remaining 4,500 ppm male rats (pH of 7.97 versus 7.66 for controls; $p < 0.05$), with occult blood[5] noted in the urine of 23 of these males. Urine samples in 10/37 surviving 4,500 ppm females tested positive for occult blood. Relative kidney weights of 1,500 and 4,500 ppm males and females and absolute kidney weights of 4,500 ppm males were statistically significantly increased (actual data were not reported).

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, but not in the other dose groups (Table 4-4). 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. Polyp-like or papillary nodules protruding into the lumen from the bladder wall were found in 41 of the 4,500 ppm male rats; 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.

[5]Blood that presents in such small quantities that it is detectible only by chemical tests or by spectroscopic or microscopic examination.

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**
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**
Lesion								
Transitional cell								
Simple hyperplasia[c]	0	0	0	12*	0	0	1	1
Nodular hyperplasia[c]	0	0	0	40*	1	0	0	5
Papillary hyperplasia[c]	0	0	0	17*	0	0	0	4
Combined hyperplasia	0	0	0	45**	1	0	1	10**
Papilloma	0	0	0	10*	0	0	0	0
Carcinoma	0	0	0	24*	0	0	0	0
Papilloma or carcinoma (combined)	0	0	0	31**	0	0	0	0
Squamous cell								
Metaplasia[c]	0	0	0	19*	0	0	0	4
Hyperplasia[c]	0	0	0	13*	0	0	0	1
Papilloma or carcinoma (combined)	0	0	0	1	0	0	0	0
Inflammatory polyp[c]	0	0	0	10*	0	0	0	0
Calculi	0	0	0	43**	0	0	0	8**

[a]TWA body weight calculated using graphically-presented body weight data in 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]The number is the sum of animals with severity grades of slight, moderate, marked, or severe.
*Statistically significant (Fisher's exact test, $p < 0.05$) as reported by study authors.
**Statistically significant (Fisher's exact test, $p < 0.05$) as determined by EPA.

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 rats 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. Simple, nodular, and papillary hyperplasias that developed in the focal area of the bladder epithelium were evident in 4,500 ppm animals. 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, the incidence of simple and nodular hyperplasia showed a dose-related increase in males and females. Treatment-related increases in the incidence of 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**
Lesion								
Ureter								
Transitional cell simple hyperplasia	1	0	0	8*	0	0	0	2
Transitional cell nodular hyperplasia	0	0	0	1	0	0	0	0
Dilatation	0	0	0	14*	0	0	0	6**
Kidney								
Renal pelvis								
Transitional cell simple hyperplasia	6	8	5	19*	3	5	12*	25*
Transitional cell nodular hyperplasia	0	1	1	21*	0	0	1	12*
Squamous metaplasia	0	0	0	2	0	0	0	0
Mineralization	9	6	10	18	12	12	18	27*
Desquamation	1	0	0	11*	0	0	0	2
Calculi	0	0	0	13*	0	0	0	3
Other								
Mineralization of corticomedullary junction	0	0	0	10*	21	2**	26	18
Mineralization of papilla	9	9	14	23*	2	6	3	12*
Papillary necrosis	0	0	0	7**	0	0	0	23*
Infarct	0	0	0	0	1	0	0	8*
Hemosiderin deposits	0	0	0	0	4	8	22*	25*
Chronic nephropathy	45	45	43	34	33	35	30	26

*Statistically significant (χ^2 or Fisher's exact test, $p < 0.05$) as reported by study authors.
**Statistically significant (Fisher's exact test, $p < 0.05$) as determined by EPA.

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, 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 biphenyl consumption data, the published results of this study were limited to kidney weight data and findings related to urinary calculi formation. 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 2,500 and 5,000 ppm 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 (by approximately 15 and 25%; $p < 0.01$) 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. Investigators described the stones in the ureter as hard, black, and composed of protein. Stones in the urinary bladder were described as hard, yellowish-white, round to oval in shape, and composed of ammonium magnesium phosphate. 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, 1,250, or 5,000 ppm biphenyl 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, 1,250, or 5,000 ppm biphenyl 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 1,250 and 5,000 ppm 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 weights of the rats receiving basal diet followed by diet containing 5,000 ppm biphenyl were significantly lower ($p < 0.001$) than that of controls (0.389 ± 22 versus 0.432 ± 30 kg). Relative kidney weights were increased in this group of biphenyl-exposed rats compared to the basal diet control group (actual data were not presented). Stones were detected only in the rats receiving 5,000 ppm biphenyl in the diet; incidences were 4/25 (kidney), 1/25 (ureter), and 3/25 (urinary bladder) in rats that had received the 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 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 ppm biphenyl, EHEN + 1,250 ppm biphenyl, and EHEN + 5,000 ppm 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, 10, 50, 100, 500, 1,000, 5,000, or 10,000 ppm for 2 years (Ambrose et al., 1960). Based on U.S. EPA (1988) reference values for body weight and food consumption in

F344 rats (averages of values for males and females), these concentrations corresponded to estimated doses of 1, 4, 8, 42, 84, 420, and 840 mg/kg-day, respectively[6]. 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 5,000 ppm biphenyl, and at 500 and 600 days in rats receiving 1,000 ppm biphenyl. A 98-day paired-feeding experiment was conducted in which control rats were provided the same amount of food that rats of the 5,000 and 10,000 ppm biphenyl groups consumed to assess whether possible differences in growth would indicate a biphenyl exposure-related toxicological response or decreased palatability. At necropsy, liver, kidney, heart, and testes weights were recorded for all groups except those receiving 10,000 ppm biphenyl in the diet. Tissues from major organs (heart, lung, liver, kidney, adrenal, spleen, pancreas, stomach, intestine, bladder, thyroid, brain, pituitary, and gonads) were examined histopathologically. In some cases, bone marrow smears were prepared. Except for one rat sacrificed prior to termination, necropies were performed only on terminal sacrifice animals (males: n = 2–13 rats/group; females: n = 2–11 rats/group).

Survival was decreased in male and female rats of the 5,000 and 10,000 ppm biphenyl exposure groups, but not at lower exposure levels. Growth rates appeared similar among controls and groups exposed to biphenyl levels ≤1,000 ppm. At the two highest exposure levels, decreased growth ranged from 8 to 48% compared to control, 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 ≤1,000 ppm, levels below those associated with decreases in food consumption, body weight, and survival (i.e., 5,000 and 10,000 ppm). Relative liver and kidney weights of female rats of the 5,000 ppm 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 rats 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. The authors concluded that there was no compound-related increase in tumor incidence. Bladder tumors were reported in male rats in most groups (controls–2/9; 10 ppm–2/8; 100 ppm–1/9; 1,000 ppm–1/9; 5,000 ppm–1/2; and 10,000 ppm–1/2) and female control rats (1/9). However, because of the small numbers of animals per group at terminal sacrifice, especially the two

[6]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.

highest dose groups where survival was only 13–33%, and because histopathological examination was limited to terminal sacrifice animals, this study was not adequate to evaluate the potential for biphenyl to induce tumors. The study identified a NOAEL of 1,000 ppm biphenyl in the diet (84 mg/kg-day) and a LOAEL of 5,000 ppm (420 mg/kg-day) 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

Biphenyl in diet (ppm)	Days on diets	Number of rats	Mean body weight (g) ± SE	Mean relative organ weight (g) ± SE			
				Liver	Kidneys	Heart	Testes
Males							
0	745	9	396 ± 24.6	2.89 ± 0.16	0.75 ± 0.02	0.32 ± 0.015	0.72 ± 0.03
10	744	8	424 ± 5.1	2.66 ± 0.06	0.70 ± 0.03	0.28 ± 0.008	0.62 ± 0.07
50	747	10	383 ± 19.8	2.84 ± 0.15	0.73 ± 0.02	0.30 ± 0.01	0.56 ± 0.06
100	752	11	394 ± 14.2	2.47 ± 0.07	0.72 ± 0.01	0.31 ± 0.008	0.67 ± 0.07
500	730	13	371 ± 15.8	3.03 ± 0.12	0.74 ± 0.02	0.31 ± 0.007	0.65 ± 0.06
1,000	746	10	366 ± 23.7	2.98 ± 0.19	0.83 ± 0.05	0.34 ± 0.012	0.60 ± 0.08
5,000	746	2	345	3.12	1.17	0.36	0.36
Females							
0	745	9	333 ± 9.4	3.11 ± 0.15	0.65 ± 0.01	0.33 ± 0.01	NA
10	744	6	369 ± 13.4	3.21 ± 0.17	0.62 ± 0.02	0.28 ± 0.07	NA
50	747	5	335 ± 16.6	2.81 ± 0.28	0.64 ± 0.02	0.31 ± 0.03	NA
100	752	11	341 ± 9.1	3.46 ± 0.74	0.62 ± 0.02	0.30 ± 0.01	NA
500	730	5	306 ± 12.5	3.51 ± 0.12	0.68 ± 0.02	0.31 ± 0.01	NA
1,000	746	5	327 ± 6.8	3.18 ± 0.10	0.65 ± 0.01	0.32 ± 0.01	NA
5,000	746	5	226 ± 25.8	4.52 ± 0.20*	1.39 ± 0.14*	0.46 ± 0.04	NA

*Statistically significant (Student's *t*-test, $p < 0.05$) as reported by study authors.

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. 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. 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. Overall, this study was too limited in duration (13-month exposure) and group size for use in evaluating the carcinogenicity of biphenyl in rats.

Sprague-Dawley rats (12/sex/group) were exposed to biphenyl in the diet for 2 years at exposure levels of 0, 100, 1,000, or 10,000 ppm (Dow Chemical Co, 1953). Based on U.S. EPA (1988) chronic reference values for body weight and food consumption in Sprague-Dawley rats (average values for combined sexes), these dietary levels are estimated to correspond to doses of 7, 73, and 732 mg/kg-day, respectively. 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. An outbreak of pneumonia 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 1,000 ppm biphenyl in the diet survived to the end of the 21st month, and none had survived by the end of the 23rd month. The authors considered the decreased survival in this group of females to have been compound-related. Eight to 30% of biphenyl concentration-related reductions in body weight gain were observed among the groups, although, in monitoring food efficiency (data not provided in report), 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). 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, incidences for tubular dilatation with severity scores ≥2 were 1/12, 6/12, 7/12, and 11/12, respectively, for males and 1/12, 3/12, 4/12, and 11/12, respectively, for females. Respective 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 in high-dose rats. The study identified a LOAEL of 1,000 ppm in the diet (732 mg/kg-day) for renal effects (renal tubular dilatation with a severity score ≥3) in Sprague-Dawley rats and a NOAEL of 100 ppm biphenyl (73 mg/kg-day). 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) conducted by JBRC, biphenyl was administered in the diet for 2 years at concentrations of 0, 667, 2,000 or 6,000 ppm corresponding to 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 (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 controls. Mean terminal body weights showed a dose-related decrease; body weights were significantly less than those of controls at 2,000 and 6,000 ppm (males: 46.9, 43.1, 42.9, and 32.4 g; females: 34.0, 32.5, 30.5, and 25.5 g, at 0, 667, 2,000, and 6,000 ppm, respectively).

Although there were no compound-related changes in hematological parameters, some clinical chemistry parameters showed marked changes in relation to biphenyl dose, including a dose-related increase in BUN that achieved statistical significance in 6,000 ppm males and females and 2,000 ppm males. In female mice, dose-related increases in activities of the plasma enzymes alkaline phosphatase (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*
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*	22.9 ± 2.7*
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*	325 ± 448*
ALT (IU/L)	32 ± 18	56 ± 46	134 ± 231*	206 ± 280*
AP (IU/L)	242 ± 90	256 ± 121	428 ± 499	556 ± 228*
LDH (IU/L)	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161*
BUN (mg/dL)	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7*

*Statistically significant (Dunnett's test, $p < 0.05$) as reported by study authors.

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 liver weight data were not presented in Umeda et al. (Umeda et al., 2005)]. Gross pathologic examinations revealed biphenyl dose-related increased incidences of liver nodules in females, but not in males (Table 4-8). The nodules were round- or oval-shaped cystic or solid masses (~3–23 mm in diameter). 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. Significantly increased incidences of basophilic cell foci were observed in 2,000 and 6,000 ppm female mice. The incidence of basophilic cell foci was significantly increased in 667 ppm male mice, but not in 2,000 or 6,000 ppm males compared to controls. 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; maximum incidence of 8%]). Liver tumor incidences in male mice showed a statistically significant decrease with increasing dose; however, the incidences were within the range of historical control data for adenomas or carcinomas in male mice (10−68%; see Table 4-8), and may reflect the higher background rate of hepatocellular tumors in male mice relative to female mice and the dose-related decrease in body weight gain (e.g., Leakey et al., 2003; Haseman and Johnson, 1996). Investigators reported statistically significantly increased incidences of desquamation of the urothelium in the renal pelvis in 6,000 ppm male and female mice, and mineralization in the inner stripe of the outer medulla of the kidney in 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[a]								
Adenoma	8/50	6/49	7/50	3/50	2/50	3/50	12/50*	10/49*
Carcinoma	8/50	8/49	5/50	4/50	1/50	5/50	7/50*	5/49
Adenoma or carcinoma (combined)	16/50	12/49	9/50	7/50**	3/50	8/50	16/50*	14/49*
Basophilic cell foci	0/50	6/49*	1/50	2/50	1/50	1/50	12/50*	6/49*
Clear cell foci	0/50	6/49*	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*	4/50	0/50	0/50	15/49*
Mineralization inner stripe–outer medulla	9/50	8/49	14/50	14/50	3/50	5/50	12/50*	26/49*

[a]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—2.5% (0–8%), adenoma/carcinoma—7.1% (2–14%). Source: Kang-Sickel (2011) email dated July 25, 2011, from Yumi Umeda, JBRC, to Connie Kang, National Center for Environmental Assessment (NCEA), Office of Research and Development (ORD), U.S. EPA.
*Statistically significant (Fisher's exact test, $p < 0.05$) as reported by study authors.
**Statistically significant (Fisher's exact test, $p < 0.05$) as determined by 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 in males, based on significantly increased incidences of hepatocellular adenomas and combined carcinomas or adenomas in the female mice receiving biphenyl from the diet (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 (n = 60) were fed diets containing 0 or 5,000 ppm biphenyl in the diet for 2 years (Imai et al., 1983). Food consumption, body weights, and survival were assessed at intervals throughout exposure. At terminal sacrifice, several organs were weighed (9–11/group). The following organs were examined for histopathological changes, in 34-37 mice/group: 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 estimated food consumption rates (U.S. EPA, 1988) and reported average terminal body weight (0.037 kg), the dose corresponding to a diet of 5,000 ppm is estimated to be 855 mg/kg-day.

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 nonneoplastic 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%]; $p > 0.05$ by Fisher's exact test). However, the lack of histopathological information concerning approximately 40% of the animals on test increases the uncertainty of these results. 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 was associated with exposure to 5,000 ppm biphenyl in the diet (estimated dose of 855 mg/kg-day) for 2 years in female ddY mice (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 generation: strain B6C3F1, designated by study authors as strain A) or male AKR mice (F1 generation: strain B6AKF1,

designated as strain B) to individual chemicals by the oral route for 18 months (NCI, 1968). [The study was subsequently published as Innes et al. (1969), but detailed results for biphenyl were not included in that publication.] 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. 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), a TWA 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).

Incidences of hepatomas, pulmonary tumors, and sarcomas in control mice and biphenyl-treated mice are summarized in Table 4-9. There were no statistically significant increases in hepatoma or pulmonary tumor incidence; however, it should be noted that the study duration of 18 months would tend to underestimate incidences associated with 24-month exposures. EPA found only the reticular cell sarcoma incidence was significantly elevated in strain B female mice but not in male mice of this strain or strain A mice of either sex. The origin of this kind of neoplasm is uncertain as three different stromal cells (follicular dendritic cells, interdigitating reticular cells, and interfollicular fibroblastic reticular cells) could give rise to reticular cell sarcoma, and special staining is needed to differentiate (Jones et al., 2001). This pathology term is not considered specific because no information on differential diagnosis was provided in the NCI (1968) report. Interpretation of the biological significance of this tumor type may also be influenced by the early-life exposure in this study, starting at 1 week of age.

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
C57BL/6 × C3H/Anf ($B6C3F_1$ or "strain A") male mice			
Controls	8/79 (10.1%)	5/79 (6.3%)	5/79 (6.3%)
Biphenyl-treated	2/17 (11.8%)	3/17 (17.7%)	1/17 (5.9%)
C57BL/6 × C3H/Anf ($B6C3F_1$ or "strain A") female mice			
Controls	0/87 (0%)	3/87 (3.4%)	4/87 (4.6%)
Biphenyl-treated	0/18 (0%)	1/18 (5.6%)	0/18 (0%)
C57BL/6 × AKR ($B6AKF_1$ or "strain B") male mice			
Controls	5/90 (5.6%)	10/90 (11.1%)	1/90 (1.1%)
Biphenyl-treated	3/17 (17.6%)	1/17 (5.9%)	0/17 (0%)
C57BL/6 × AKR ($B6AKF_1$ or "strain B") female mice			
Controls	1/82 (1.2%)	3/82 (3.7%)	4/82 (4.9%)
Biphenyl-treated	0/17 (0%)	0/17 (0%)	4/17 (23.5%)*

[a]Tumor incidences were tallied from those mice for which histopathological examinations were performed.
*Statistically significant (Fisher's exact test, $p < 0.05$) as determined by EPA.

Source: NCI (1968).

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 from 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 were 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, 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 standard deviation [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. Body weights and 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 histopathological 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 histopathological lesions for combined male and female mice only; no statistical analyses were conducted.

Source: Sun (1977a).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

Pregnant female Wistar rats (18–20/ group) were administered 0, 125, 250, 500, or 1,000 mg/kg-day biphenyl in corn oil by gavage on gestation days (GDs) 6–15 (with GD 1 defined as the the day the evidence of copulation was observed) (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 necropsy included the number of corpora lutea, fetal weights and viability, and resorptions; fetal sex was apparently not determined. Two-thirds of the live fetuses/litter were examined for skeletal development and the rest were examined for the presence of visceral abnormalities.

At 1,000 mg/kg-day, 5 of 20 high-dose dams died prior to sacrifice, and there was a 10% decrease from control in body weight in the remaining dams in that group (data not provided). Doses ≤500 mg/kg-day produced no clinical signs of maternal toxicity or evidence of treatment-related effects on maternal weight gain. The number of dams without live fetuses was significantly increased at 1,000 mg/kg-day; of the surviving dams, five were found not pregnant and one had seven resorption sites but no live fetuses (Table 4-11). Mean numbers of corpora lutea and live fetuses per pregnancy in the remaining pregnant 1,000 mg/kg-day dams were similar to those of controls and dams of other dose levels.

The incidence of anomalous fetuses and litters bearing anomalous fetuses, including wavy ribs, extra ribs, missing and unossified sternebrae, or delayed calvarium ossification, generally increased with dose. When data from the high-dose (1,000 mg/kg-day) group were dropped because of frank maternal toxicity at that dose, missing or unossified sternebrae was the only endpoint that showed a statistically significant increasing trend with dose (Cochran-Armitage test).

As noted in EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), a significant, dose-related increase in a variation (e.g., delayed ossification) should be evaluated as a possible indication of developmental toxicity, although an assessment of the biological significance of such variations should take into consideration knowledge of the developmental stage, background incidence of certain variations, other strain- or species-specific factors, and maternal toxicity. Other information that would help in interpreting the biological significance of anomalies in Khera et al. (1979), however, were not available. In light of the finding of a statistically significant increasing trend of missing or unossified sternebrae with dose and consideration of this anomaly as more severe than the other anomalies identified, EPA identified a LOAEL of 500 mg/kg-day for increased incidence of fetuses with missing and unossified sternebrae and a NOAEL of 250 mg/kg-day.

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 (9.7%)	22/236 (9.3%)	22/213 (10.3%)	35/199 (17.6%)	25/107 (23.4%)
Anomalous litters/number examined*	8/16 (50%)	11/20 (55%)	13/18 (72%)	15/18 (83%)	6/9 (67%)
Anomalies, number (percent) of fetuses affected					
Wavy ribs, uni- and bilateral	3 (1.7%)	7 (3.0%)	9 (4.2%)	8 (4.0%)	5 (4.7%)
Extra ribs, uni- and bilateral	9 (5.1%)	12 (5.1%)	9 (4.2%)	15 (7.5%)	6 (5.6%)
13th rib, small sized	1 (0.6%)	1 (0.4%)	2 (0.9%)	1 (0.5%)	0 (0.0%)
Sternebrae, missing or unossified*	4 (2.3%)	3 (1.3%)	4 (1.9%)	16 (8.0%)	17 (15.9%)
Calvarium, delayed ossification	0 (0.0%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	8 (7.5%)
Miscellaneous	1 (0.6%)	1 (0.4%)	1 (0.5%)	0 (0.0%)	0 (0.0%)

[a]Five dams died prior to scheduled sacrifice; five were not pregnant at term; and one 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.
*Statistically significant trend (Cochran-Armitage trend test, $p < 0.05$) as determined by EPA, after dropping the highest dose because of frank maternal toxicity.

Source: Khera et al. (1979).

Dow Chemical Co (1953) reported the results of a multigenerational study conducted by the Stanford Research Institute in which groups of 4-month-old male and female Long-Evans rats (three males and nine females/group) were fed diets containing 0, 100, 1,000, or 10,000 ppm biphenyl. Based on U.S. EPA (1988) subchronic reference values for body weight and food

consumption in male and female Long-Evans rats, these dietary concentrations are estimated to correspond to doses of 9, 89, and 887 mg/kg-day, respectively, for the males and 10, 101, and 1,006 mg/kg-day, respectively, for the females. For breeding, three females were placed together with one male. Following the breeding phase, females were separated and the number of litters cast, number of days between mating and delivery, and 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 dose group were subjected to gross pathologic examinations.

There were no significant differences between controls and 100 and 1,000 ppm biphenyl 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 10,000 ppm biphenyl group of F0 and F1 females was observed (6/9, 7/9, and 8/9 confirmed pregnancies for the three successive generations of 10,000 ppm biphenyl groups versus 8/9, 9/9, and 8/9 confirmed pregnancies for controls). Averaged for F1, F2, and F3 pups combined, the 10,000 ppm biphenyl group exhibited a 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 (34 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 reported evidence of a cumulative effect over the three generations. The study authors suggested the possibility that the decreased fertility, smaller litter size, and reduced rate of growth in the 10,000 ppm biphenyl group may have been associated with unpalatability and resultant decreased food intake; however, food consumption data were not reported. Further, palatability is unlikely to have been the cause of all observed effects since gavage dosing at a similar dose level produced maternal and fetal toxicity in the Khera et al. (1979) study. Overall, this report provides evidence of reproductive toxicity (decreased fertility, smaller litter size, and reduced pup growth rate) at an estimated dose of 887 mg/kg-day—similar to the dose that caused frank maternal toxicity in the Khera et al. (1979) study. It should be noted that the design of this study did not include the more extensive evaluation of reproductive endpoints that would be included in studies conducted using current study protocols.

Ambrose et al. (1960) examined the reproductive toxicity of biphenyl in two experimental series. In the first experiment, weanling albino rats were administered 0 or 1,000 ppm biphenyl (5 males and 10 females/group) or 5,000 ppm 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 1,000 ppm biphenyl (4 males and 8 females/group) or 5,000 ppm 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), these dietary levels correspond to estimated doses of 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. Although the authors concluded that the compound had no significant effect on reproduction, the reported data for number of rats casting litters, total born, and range of litter size (Table 4-12) were insufficient to support a full evaluation of the association between dietary exposure to biphenyl and reproductive deficits.

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

Experimental series	Diet (ppm)[c]	Dams with litters	Total offspring	Litter size (range)
First[a]	Control	9/10	59	3–9
	1,000	10/10	67	2–10
	5,000	8/9	53	3–9
Second[b]	Control	8/8	64	5–13
	1,000	6/8	63	3–10
	5,000	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]1,000 ppm = 105 mg/kg-day and 5,000 ppm = 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, 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.

In a study by Sun (1977b), 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. Average weight loss for the rabbits receiving purified and technical biphenyl was 45 and 172 g, 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).

In a preliminary study, five adult rats (sex and strain unspecified) were administered 10,000 ppm biphenyl in the diet 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 4-hydroxybiphenyl and its glucuronide. Similar effects were observed in male and female rats receiving 5,000 ppm, but not 500 ppm, biphenyl in the diet,.

A follow-up study employed 42 rats/sex/group and biphenyl dietary levels of 1,000, 2,500, or 5,000 ppm. Biphenyl doses are estimated to be 83.7, 209, and 419 mg/kg-day, 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. Consistent with the preliminary study findings, the rats of the 5,000 ppm group in the follow-up study exhibited gradual increases in urine volume and sulfosalicylic acid-precipitable sediment and decreases in both parameters during postexposure recovery. These effects were less pronounced in the 2,500 ppm group and absent in the 1,000 ppm group. At 5,000 ppm, kidney lesions were noted in 1/5 males (several small cysts and dilated tubules in the medulla and inner cortex) and 2/5 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 2,500 ppm 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 5,000 ppm biphenyl 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 kidney histopathology. 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 (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. 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. Change in kidney weight and cellular architecture in Wistar rats exposed to biphenyl

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 5,000 ppm 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. Treatment was associated with 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 noted 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 5,000 ppm 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 10,000 ppm biphenyl in the diet 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 + 10,000 ppm 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 10,000 ppm biphenyl in the diet 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, 1,250, or 5,000 ppm biphenyl for 34 weeks (Shiraiwa et al., 1989). 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, corresponding doses are estimated to have been approximately 0, 60, and 248 mg/kg-day, respectively. 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.

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 drinking water for 4 weeks followed by diets containing either 0 or 5,000 ppm 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 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 5,000 ppm biphenyl in the diet 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 bladder, 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 C. Mechanistic studies of biphenyl effects on the urinary bladder, a principal target of biphenyl toxicity, and genotoxic potential are briefly discussed below.

4.5.1. Effects on the Urinary Bladder of Rats

Mechanistic studies have been performed in F344 rats to investigate the relationship between calculi formation in the urinary bladder and bladder tumor induction in male rats. Ohnishi et al. (2000a), Ohnishi et al. (2001), and Ohnishi et al. (2000b) proposed that gender differences in urinary conditions (including pH and potassium concentrations) and kidney sulphatase activity 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. Information from available mechanistic studies is summarized in Appendix C.

4.5.2. Genotoxicity

The genotoxicity studies of biphenyl and its metabolites are summarized in Appendix C (Tables C-2 and C-3). A review of the available data suggests that biphenyl may have some capability of inducing genetic damage under certain conditions. Bacterial mutagenicity assays are uniformly negative, even with metabolic activation; however, several in vitro mammalian cell assays were able to detect weak evidence of mutagenicity with activation (Glatt et al., 1992; Wangenheim and Bolcsfoldi, 1988). Indications of the ability to induce chromosomal aberrations were also observed with the addition of metabolic activation (Sofuni et al., 1985), although this was accompanied by cytotoxicity in one study without metabolic activation (Rencüzoğullari et al., 2008). In addition, evidence of deoxyribonucleic acid (DNA) strand breaks was observed in mice in several organs, including the stomach, blood, liver, bone marrow, kidney, bladder, lung, and brain (Sasaki et al., 2002; Sasaki et al., 1997). Micronuclei were observed in primary human lymphocytes (Rencüzoğullari et al., 2008), but were not found in another study in mouse bone marrow (Dow Chemical Co, 2007). Chromosomal aberrations were not observed following inhalation exposures in rats (Dow Chemical Co, 1976).

There are indications that the metabolites of biphenyl may be more genotoxic than the parent compound when the metabolites are directly tested in assay systems. Genotoxicity results for the major metabolite, 4-hydroxybiphenyl, and a minor metabolite, 2-hydroxybiphenyl (i.e.,

o-phenylphenol, or OPP), can be found in Appendix C (Table C-3). Thus, it is possible that the genotoxic potential in any given system or organism is directly related to the proportion that these metabolites are formed in that system.

It is unknown if reports of DNA damage following exposure to biphenyl are caused by a direct reaction of metabolites with DNA or by indirect damage from cytotoxicity, ROS generated from redox cycling of hydroquinone metabolites, or some combination of these mechanisms. Biphenyl in an activated system was not investigated for its ability to form DNA-reactive metabolites, but in studies of DNA adduct formation using the metabolites, most were negative (Kwok et al., 1999; Smith et al., 1998) except for one study of high doses applied to skin (Pathak and Roy, 1993). However, several reports indicate that genetic damage often occurred only after high doses that were accompanied by decreased cell survival or was concurrent with redox cycling following metabolism of 2-hydroxybiphenyl, a minor metabolite of biphenyl (see Appendix C). One study that directly tested the mutagenicity of the major metabolite, 4-hydroxyquinone, in the Salmonella Ames assay was positive (Narbonne et al., 1987), but no other investigations of this metabolite were located. In summary, there is not enough evidence to conclude that biphenyl is mutagenic or can react directly with DNA. The available genotoxicity database suggests that most indications of genotoxicity following biphenyl exposure are likely to be secondary responses resulting from oxidative damage and cytotoxicity.

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[a]

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	Not determined	Not determined	Lower average plasma BUN levels in all exposed groups (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 three highest dose groups received lower doses for exposure wks 1–2, followed by the final dose for the remaining time.	Umeda et al. (2004a)
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)
Rat, Wistar (50/sex/group)	Diet	M: 0, 165, or 353 F: 0, 178, or 370 75 wks	M: Not determined F: Not determined	M: 165 F: 178	Formation of kidney stones associated with pyelonephritis in both sexes		Shiraiwa et al. (1989)

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

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 (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	Necropsies were performed on terminal sacrifice animals only (n = 2–13 animals/group).	Ambrose et al. (1960)
Rat, albino (male, 8/group)	Diet	0, 250, or 450 13 mo	Not determined	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 the 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[a]

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. Increased incidence of liver tumors (adenoma and carcinoma) in females.		Umeda et al. (2005)
Mouse, ddY (female, 60/group)	Diet	0 or 855 2 yrs	855	Not determined	No adverse effects observed at dose tested	Results were reported only for 34–37/group.	Imai et al. (1983)
Mouse, 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	Not determined	Reticular cell sarcoma incidence significantly elevated in strain B female mice, but not in male mice of this strain or strain A mice of either sex	Two strains of F1 hybrid mice were produced by mating female C57BL/6 mice with either male C3H/Anf mice (strain A) or male AKR mice (strain B)	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	Not determined	Not determined	Not determined		Monsanto (1946)[a]
Monkey, Rhesus (2 males/group; 1 female/group)	Diet	0, 0.01, 0.1, or 1% for 1 yr	Not determined	Not determined	Not determined	The study 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[a]

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 dead or resorbed fetuses Offspring: Increased incidence of fetuses with missing and unossified sternebrae		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: Not determined F: 101	M: Not determined F: 1,006	M: Not determined F: Decreased fertility and litter size; reduced offspring body weight.	The authors suggested that effects seen in the high-dose group were associated with unpalatability and resultant decreased food intake; however, food consumption data were not provided to support this interpretation.	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 d prior to mating Experiment 2: 11 d prior to mating	Not determined	Not determined	Not determined	Authors presented tabulated data and concluded that the compound had no significant effect on reproduction.	Ambrose et al. (1960)

[a]Note: Other studies of subchronic duration that examined the effects of biphenyl on the urinary tract only (Shibata et al., 1989b; Shibata et al., 1989a) 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.
[b]Report was not peer reviewed.

F = female; M = male

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: Not determined Rat: Not determined	Rabbit: Not determined Rat: 300	Rabbit: Not determined 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: Not determined Rat: Not determined	Rabbit: Not determined Rat: 40	Rabbit: Not determined Rat: Mortality (1/6), acute emphysema, congestion, edema, bronchitis, lobular pneumonia, and multiple pulmonary abscesses	
Mouse (12/group) Rat, Sprague-Dawley (4/group)	5 mg/m^3 (7 hrs/d, 5 d/wk) 62 d over 92-d period	Mouse: Not determined Rat: Not determined	Mouse: 5 Rat: Not determined	Mouse: Mortality (2/12); upper respiratory tract irritation (acute emphysema, congestion, edema, bronchitis, lobular pneumonia, and multiple pulmonary abscesses) Rat: Not determined	
Mouse, CDI (50/sex/group)	0, 157.7, or 315.3 mg/m^3 (7 hrs/d, 5 d/wk), 13 wks	Not determined	157.7	Histopathological lung, liver, and kidney lesions (congested and hemorrhagic lungs, tracheal hyperplasia, and congestion and edema in the liver and kidney) in both sexes	Sun (1977b)[a]

[a]Report was not published.

4.6.1. Oral

The primary targets of toxicity of ingested biphenyl in experimental animals are the kidney, urinary bladder, liver, and developing fetus. Decreased body weight has also been associated with oral biphenyl exposure. No information was located regarding possible associations between oral exposure to biphenyl and health outcomes in humans.

Chronic oral studies identified the kidney as one of the noncancer targets of biphenyl in both rats and mice. Exposure to biphenyl in the diet for 2 years produced a range of histopathological changes in the kidney in F344 rats (Umeda et al., 2002). Mineralization of the papilla (part of the renal medulla) showed a dose-related increase in both male and female rats; papillary necrosis was observed in both sexes of rats at the high dose only. Papillary mineralization can be found in association with papillary necrosis (Bach and Nguyen, 1998), and the histopathologic changes in the medulla overall suggest a continuum of increasing severity of damage with increasing biphenyl dose. Effects in the papillary region of the medulla were supported by dose-related histopathologic changes in the renal pelvis of male and female rats in the Umeda et al. (2002) bioassay, including mineralization, transitional cell hyperplasia (simple and nodular), desquamation, and calculus formation. A dose-related increase in the incidence of hemosiderin deposits was observed in female rats, but not in male rats at any dose level. Hemosiderin, an iron-protein complex that may be present as a product of hemoglobin degradation, can arise from various conditions (Jennette et al., 2007). Without information in Umeda et al. (2002) on severity and location of hemosiderin within the kidney, the biological significance of this endpoint is unclear. Kidney findings were consistently observed in other studies in rats, including tubular dilation or mild tubuli degeneration in albino and Sprague-Dawley rats (Ambrose et al., 1960; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953) and calculi formation in the renal pelvis in Wistar and albino rats (Shiraiwa et al., 1989; Ambrose et al., 1960). Dose-related pathological changes in the kidney in BDF_1 mice following 2-year dietary exposure to biphenyl included desquamation of the renal pelvis and mineralization of the medulla (Umeda et al., 2005). A dose-related increase in BUN levels in mice in this study (Umeda et al., 2005) provides evidence of biphenyl-induced functional disruption of the kidney. Imai et al. (1983) did not find histopathological changes in the kidney of ddY mice exposed to biphenyl in diet for 2 years; however, only ~60% of the animals were subjected to pathological examination in this study. There is a hazard potential for kidney toxicity based on consistent evidence of biphenyl-induced kidney toxicity in studies in rats and some support from studies in mice.

Urinary bladder toxicity associated with oral exposure to biphenyl was observed in rats only. Increased incidences of urinary bladder hyperplasia and calculi or stones were observed in male and female F344 rats exposed to biphenyl in the diet (378 and 438 mg/kg-day, respectively) for 2 years (Umeda et al., 2002) and in male and female Wistar rats exposed to biphenyl in the diet (353 and 370 mg/kg-day, respectively) for up to 75 weeks (Shiraiwa et al., 1989). In a

subchronic study by Shibata et al. (1989b), increases in BrdU labeling index and simple hyperplasia in urinary bladder epithelium were observed in male F344 rats given biphenyl in the diet (500 mg/kg-day) for 4 weeks. Ambrose et al. (1960) and (Dow Chemical Co, 1953) did not find lesions in urinary bladder in albino or Sprague-Dawley rats exposed to biphenyl in the diet for two years; however, both studies used relatively small group sizes and provided limited necropsy data. Biphenyl did not induce changes in the urinary bladder in mice (Umeda et al., 2005; Imai et al., 1983). There is a hazard potential for urinary bladder toxicity from biphenyl exposure based on evidence of calculi formation and epithelial lesions in the urinary bladder of rats. Because urinary bladder toxicity was not found in a second species, the evidence for hazard potential is weaker than for the kidneys.

Liver toxicity, including histopathological changes and increased liver weight and serum liver enzymes, were observed in studies of mice and rats. Relative liver weight was increased by more than 10% in female albino and Sprague-Dawley rats exposed to 420 and 732 mg/kg-day biphenyl for 2 years, respectively (Ambrose et al., 1960; Dow Chemical Co, 1953), and in Rhesus monkeys exposed to 1% biphenyl in the diet for 1 year (Dow Chemical Co, 1953). The only histopathological change observed in rats was moderate degeneration of parenchymal hepatocytes within 2 months followed by regenerative hyperplasia and nuclear hypertrophy that persisted to 13 months in male albino rats exposed to ≥250 mg/kg-day biphenyl (Pecchiai and Saffiotti, 1957). Liver toxicity was not reported in F344 rats exposed to biphenyl in the diet up to 438 mg/kg-day for 2 years (Umeda et al., 2002). Differences in response in the two studies may be due to differences in strain susceptibility. In BDF_1 mice, relative liver weight of female mice exposed to 134–1,420 mg/kg-day biphenyl in the diet for 2 years was increased by 1.3–1.6-fold (Umeda et al., 2005); biphenyl exposure did not affect liver weight in male mice. Histopathological changes included enlarged centrilobular hepatocytes filled with eosinophilic granules identified as peroxisomes in BDF_1 mice exposed to 2,989 mg/kg-day biphenyl in the diet for 13 weeks (Umeda et al., 2004a) and basophilic foci in female BDF_1 mice exposed to biphenyl in the diet (≥414 mg/kg-day) for 2 years (Umeda et al., 2005). Significantly increased plasma enzyme levels (AST, ALT, AP, and LDH) were observed primarly in female BDF_1 mice exposed to biphenyl in the diet for 2 years (Umeda et al., 2005). No liver toxicity was found in female ddY mice exposed to 855 mg/kg-day biphenyl for 2 years (Imai et al., 1983) based on histopathological examination of ~60% of the animals (34 of 60). In summary, biphenyl exposure resulted in increased liver weight and histopathological changes of the liver in mice and rats and increased liver weight in monkeys; however, liver toxicity was not observed consistently across different strains of rats and mice or across sexes. Based on these findings, liver toxicity may be a hazard potential from biphenyl exposure.

In the only available oral developmental toxicity study of biphenyl (Khera et al., 1979), the incidence of anomalous fetuses and litters bearing anomalous fetuses (including wavy ribs, extra ribs, missing and unossified sternebrae, or delayed calvarium ossification) generally

increased with dose. When the anomalies were considered individually, only the incidence of missing or unossified sternebrae exhibited an increasing trend with dose. As noted in EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), a significant, dose-related increase in a variation (e.g., delayed ossification) should be evaluated as a possible indication of developmental toxicity, although an assessment of the biological significance of such variations should take into consideration knowledge of the developmental stage, background incidence of certain variations, other strain- or species-specific factors, and maternal toxicity. Carney and Kimmel (2007) observed that the biological significance of skeletal variations that seem to be readily repairable via postnatal skeletal remodeling should be interpreted in the context of other maternal and fetal findings, information on normal skeletogenesis patterns, mode of action of the agent, and historical control incidence. The Khera et al. (1979) study showed a 10% decrease in body weight gain and frank maternal toxicity in dams at the high dose of 1,000 mg/kg-day (increased mortality [5/20 versus 0/18 in controls]), but not at doses of 125, 250, or 500 mg/kg-day. Therefore, the increasing trend of fetuses with missing or unossifed sternebrae at ≤500 mg/kg-day cannot be attributed to maternal toxicity. In summary, findings from a single developmental toxicity study (Khera et al., 1979) provide evidence that biphenyl may directly target skeletal development in Wistar rats independent of maternal toxicity; however, no other developmental toxicity studies are available to confirm these findings. Based on these findings, there may be a hazard potential for developmental toxicity from biphenyl exposure.

Reproductive effects of biphenyl were evaluated in one- and three-generation reproductive toxicity study (Ambrose et al., 1960; Dow Chemical Co, 1953). There was some indication in Dow Chemical Co (1953) of reduced fertility and decreased pup growth at an estimated oral dose of 887 mg/kg-day, similar to the dose used in a developmental toxicity study (Khera et al., 1979) that caused maternal toxicity (reduced survival and body weight gain). Ambrose et al. (1960) reported limited findings and concluded that biphenyl had no significant effect on reproduction in albino rats exposed to biphenyl in the diet at doses up to 525 mg/kg-day. Overall, the available reproductive toxicity studies in rats (Ambrose et al., 1960; Dow Chemical Co, 1953) did not fully evaluate effects of biphenyl exposure on reproductive function as would studies conducted using current study protocols, but suggested that possible reproductive toxicity would occur at doses similar to the dose associated with frank maternal toxicity in another developmental toxicity study.

Decreased body weight gain associated with biphenyl exposure was observed in both rats and mice. Following a 2-year dietary exposure to biphenyl, a >10% decrease in body weight relative to controls was reported in F344 rats of both sexes (males—378 mg/kg-day; females—438 mg/kg-day) (Umeda et al., 2002) and in BDF_1 mice in both sexes (males—291 mg/kg-day; females—≥414 mg/kg-day) (Umeda et al., 2005). A 75-week study in Wistar rats also found a >10% body weight decrease in males at doses ≥165 mg/kg-day and in females at doses ≥178

mg/kg-day (Shiraiwa et al., 1989). Shorter-duration oral exposure (13 weeks) of mice to biphenyl at higher dietary concentrations (estimated doses ≥1,500 mg/kg-day) was also associated with >17% decreased body weight (Umeda et al., 2004a). Ambrose et al. (1960) and Dow Chemical Co (1953) reported >10% reduced body weight gain, but the authors attributed low body weight to low palatability of the feed. In summary, decreased body weight gain appears to be associated with oral exposure to biphenyl.

4.6.2. Inhalation

The toxicity of inhaled biphenyl has received less investigation than ingested biphenyl. An epidemiological study of workers engaged in the production of biphenyl-impregnated paper (Seppalainen and Hakkinen, 1975; Häkkinen et al., 1973; Häkkinen et al., 1971) provides some evidence of liver damage (including elevated levels of serum AST and ALT) and effects on the central and peripheral nervous systems (including abnormal EEGs and ENMGs). In a study of a different facility manufacturing biphenyl-impregnated paper prompted by the finding of three cases of Parkinson's disease at that facility, an elevated RR of Parkinson's disease among biphenyl workers was reported (Wastensson et al., 2006). The occurence of Parkinson's disease was not confirmed by the earlier study by Seppalainen and Hakkinen (1975), despite workplace concentrations that appeared to be considerably higher. The workplace conditions reported for these studies (Wastensson et al., 2006; Seppalainen and Hakkinen, 1975; Häkkinen et al., 1973; Häkkinen et al., 1971) suggested that inhalation represented the predominant route of exposure and that existing occupational exposure limits had been exceeded, 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 55 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 methodology 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 urinary bladder is a target of biphenyl toxicity in the rat, and histopathological lesions in this organ appear to be related to the formation of urinary bladder calculi induced by biphenyl exposure. Mode-of-action information related to the role of calculi formation in the

induction of urinary bladder toxicity is described in Section 4.7.3.1. The mode of action for biphenyl-induced toxicity in the kidney, another organ in the urinary system, has not been investigated. Bioassay data suggest that a mode of action involving calculi formation does not fully explain kidney lesions induced by biphenyl; kidney lesions were found in mice exposed to biphenyl in the diet for 104 weeks without calculi formation (Umeda et al., 2005). Further, the incidences of kidney histopathologic lesions in male and female rats exposed to biphenyl in the diet for 104 weeks were similar (Umeda et al., 2002), whereas the incidence of calculi in the kidney was lower in females than males (i.e., 3/50 versus 13/50 in the high-dose groups, respectively).

Mode-of-action information related to biphenyl-induced liver toxicity is limited to the proposed involvement of peroxisome proliferation-activated receptors (PPARs). Evaluation of the evidence for a proposed PPAR mode of action is provided in Section 4.7.3.2.

Mechanistic studies provide some information on the induction of decreased body weight gain by biphenyl. A possible mode of action is suggested by an in vitro study, where biphenyl can act as an uncoupler of respiration (Nishihara, 1985).

There is no mode-of-action information on the toxicity of biphenyl to the developing fetus or reproductive system.

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" based on increased incidence of urinary bladder tumors (transitional cell papillomas and carcinomas) in male F344 rats (Umeda et al., 2002) and liver tumors (hepatocellular adenomas and carcinomas) in female BDF_1 mice (Umeda et al., 2005) exposed to biphenyl in the diet for 104 weeks, as well as information on mode of carcinogenic action. The carcinogenic potential of biphenyl in humans has not been investigated.

As emphasized in the Cancer Guidelines (U.S. EPA, 2005a), selection of the cancer descriptor followed a full evaluation of the available evidence. The carcinogenicity evidence for biphenyl could be considered a borderline case between two cancer descriptors—"suggestive evidence of carcinogenic potential" and "likely to be carcinogenic to humans." The descriptor of "suggestive evidence of carcinogenic potential" is appropriate when a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion, given "an extensive database that includes negative studies in other species," and that "additional studies may or may not provide further insights." The database for biphenyl includes studies in rats and mice that did not show clear evidence of carcinogenicity (Shiraiwa et al., 1989; Imai et al., 1983; NCI, 1968; Ambrose et al., 1960; Dow Chemical Co, 1953), but that were conducted in different strains, and also limited in large part in design, conduct, or reporting

of results. These studies were therefore considered less informative for evaluating the carcinogenicity of biphenyl than the studies by Umeda et al. (2005) and Umeda et al. (2002). The range of evidence regarding each tumor type is described further in Section 4.7.2.

Exposure to biphenyl produced a positive tumor response at more than one site (urinary bladder and liver) and in more than one species (rat and mouse), corresponding most closely to one of the examples in the Cancer Guidelines (U.S. EPA, 2005a) for the descriptor of "likely to be carcinogenic to humans;" i.e., "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans." However, as discussed further below, mechanistic data for urinary bladder tumors and limitations in liver tumor data better support the descriptor of "suggestive evidence of carcinogenic potential" for biphenyl.

Mode-of-action information indicates that 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 in male F344 rats will not occur without the development of calculi, and that the induction of these tumors by biphenyl is specific to male rats. Gender-specific differences in urinary conditions such as pH and potassium concentrations appear to play a role in the differences in calculi formation and composition. 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). Overall, the mode-of-action analysis supports the conclusion that biphenyl should not pose a risk of urinary bladder tumors in humans at exposure levels that do not cause calculi formation.

Liver tumors induced by dietary exposure to biphenyl for 104 weeks occurred in female BDF_1 mice only. In contrast, the incidence of liver tumors in male mice decreased with increasing exposure (Umeda et al., 2005). The decreased incidences were still within the range of historical controls, and similar decreased trends in liver tumors that were associated with decreased body weight gain in $B6C3F_1$ mice, as also occurred in the BDF_1 mice exposed to biphenyl, have been judged not to demonstrate anticarcinogenicity [e.g., Leakey et al. (2003); Haseman and Johnson (1996)]. Mechanistic data to support a mode of action for biphenyl-induced liver tumors in the mouse are not available (see Section 4.7.3.2). In the absence of information to indicate otherwise, the development of liver tumors in female BDF_1 mice with chronic exposure to biphenyl (Umeda et al., 2005) is assumed to be relevant to humans. EPA acknowledges that the relative susceptibility of some mouse strains to liver tumors and the somewhat high and variable background incidence of this tumor contribute to controversy in the use of mouse liver tumor data in risk assessment (e.g., King-Herbert and Thayer (2006)). According to historical control data from JBRC, the institute that conducted the mouse bioassay published by Umeda et al. (2005), the mean incidences of liver tumors (hepatocellular adenoma

or carcinoma) in male and female control BDF_1 mice are 32.2 and 7.1%, respectively. These incidences are 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.

In summary, while the cancer descriptor "likely to be carcinogenic to humans" appears plausible and the positive evidence of tumors at two sites in two species raises a concern for carcinogenic effects in humans, this assessment acknowledges: (1) the lack of evidence for either tumor type in a second study, sex, strain, or species, and (2) the existence of a mode of action for urinary bladder tumors, specific to the male rat, establishing these tumors as a high-dose phenomenon closely related to the formation of urinary bladder calculi. Recognizing that each cancer descriptor covers a continuum of evidence, this assessment concludes that biphenyl shows "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. Studies in rats, rabbits, and guinea pigs demonstrate that biphenyl is rapidly and extensively absorbed by the oral route of exposure, and an in vitro model using human skin provides evidence of dermal absorption of biphenyl (DuPont, 2005). Qualitative evidence for absorption of inhaled biphenyl comes from inhalation toxicity studies in rats and mice that reported systemic (liver and kidney) effects following inhalation exposure to biphenyl for 46–90 days (Sun, 1977a; Deichmann et al., 1947; Monsanto, 1946). 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 human absorption by these routes. Therefore, based on the observation of systemic tumors following oral exposure and limited qualitative evidence for inhalation and dermal absorption, 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.

Urinary bladder tumors were found in F344 male rats in a well-designed 2-year cancer bioassay by Umeda et al. (2002). This is a rare tumor type, not having been observed in historical control male F344 rats of the JBRC or the National Toxicology Program (NTP)—1,148 and 1,858 rats, respectively, as reported by Umeda et al. (2002). Although the other available bioassays evaluated exposure ranges comparable to those used by Umeda et al. (2002), they did not report increased urinary bladder tumors. However, these other studies could not confirm or contradict these findings due either to smaller group sizes or shorter effective exposure durations; they were also conducted in different rat strains than the Umeda et al. (2002) study. In the 75-week dietary study in Wistar rats (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. The duration, being much shorter than the standard 104-week bioassay, may not have been sufficiently long to observe late-occurring tumors. Ambrose et al. (1960) exposed albino rats to biphenyl in the diet at concentrations ranging from 10 to 10,000 ppm for 2 years; urinary bladder tumors occurred in most groups. Because of decreased survival in rats exposed to 5,000 or 10,000 ppm and the evaluation of histopathology only for rats surviving to study termination (as few as two per group at the higher doses), however, this study was not adequate for evaluation of the tumorigenic potential of biphenyl. In the 2-year dietary study of biphenyl conducted by Dow Chemical Co (1953) in Sprague-Dawley rats (12/sex/group), a pneumonia outbreak (resulting in deaths of all control male rats by the end of 1 year), relatively small group sizes, and decreased survival may have impaired the ability to detect late-developing tumors. Overall, the evidence for urinary bladder tumors shows differing, as opposed to conflicting, results.

Evidence concerning liver tumors includes positive findings in one sex of one species (i.e., female BDF_1 mice) from a well-conducted 2-year dietary study by Umeda et al. (2005). Male mice in this study showed a statistically significant decreasing trend in liver tumor incidence with increasing dose, but the incidences at all dose levels were within the range of historical controls for the laboratory. There was no liver tumor response in either sex of $B6C3F_1$ or $B6AKF_1$ mice (NCI, 1968), but these evaluations were carried out at a lower exposure than those used by Umeda et al. (2005), for a shorter duration (18 rather than 24 months), and with treated groups of no more than 18 animals. There was no observed liver tumor response in female ddY mice (Imai et al., 1983)—males were not tested—with exposure at a level intermediate to the higher exposures tested by Umeda et al. (2005). Umeda et al. (2005) suggested that the difference in response between the two studies might be due to differences in susceptibility between the two mouse strains, but specific support for this hypothesis is not available. Overall, the evidence for liver tumors shows differing, as opposed to conflicting, results.

The 18-month NCI (1968) bioassay showed a statistically significant elevation in the incidence of reticular cell sarcoma in treated $B6AKF_1$ female mice, but not in $B6C3F_1$ female

mice or B6C3F$_1$ or B6AKF$_1$ male mice. Although this bioassay was unique among those available in starting exposure during early life at 1 week of age [i.e., versus 6 weeks for Umeda et al. (2005)], specific support for early life susceptibility to sarcomas in response to biphenyl exposure is not available. In light of the inconsistency in this finding across mouse strains and sexes in the NCI (1968) study and the lack of confirmation in other studies in mice at higher exposures, the biological significance of the elevated incidence of reticular cell sarcoma in female mice is unclear.

The evidence for genotoxicity of biphenyl and its metabolites is reviewed in Appendix C (Tables C-2 and C-3) and is summarized in Section 4.5.2. The in vitro evidence does not indicate that biphenyl is mutagenic; however, in vivo data suggest that biphenyl metabolites that are capable of redox cycling may induce genetic damage resulting from oxidative damage and cytotoxicity.

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 supported by the species and gender specificity of calculi and tumor development. Urinary bladder calculi were observed in female rats only at 4,500-ppm biphenyl in the diet and at a lower incidence (8/50; 16%) than in male rats; no urinary bladder transitional cell papillomas or carcinomas were observed in any female rats (Umeda et al., 2002). The available evidence suggests that differences in physical properties and chemical composition of calculi in male and female rats account for the gender difference in development of urinary bladder tumors (Umeda et al., 2002; Ohnishi et al., 2000b). Urinary bladder calculi in male rats are formed by irreversible chemical reactions; these calculi have been described as triangular, pyramidal, or cubical in shape, 0.3–1 cm in size, and composed primarily of potassium 4-hydroxybiphenyl-O-sulphate. In contrast, urinary bladder calculi in female rats are of homogeneous size, spheroidal in shape, 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). The calculi formed in female rats may undergo reversible hydroxylation reaction and are less stable than those formed in males (Ohnishi et al., 2000b). Umeda et al. (2005) suggested that the physical characteristics of the calculi in male rats lead to mechanical damage to the urinary bladder epithelium not induced by calculi in female rats and, hence, to tumor formation. 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).

Gender differences in urinary conditions of the rat (including 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 (Ohnishi et al., 2001; Ohnishi et al., 2000a; Ohnishi et al., 2000b). 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). 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). Ohnishi et al. (2000b) fed biphenyl, biphenyl and potassium chloride (KCl), biphenyl and sodium bicarbonate ($NaHCO_3$), or biphenyl and potassium bicarbonate ($KHCO_3$) to male F344 rats for 13 weeks. Urine crystals were found only in rats coadministered biphenyl and $KHCO_3$. These observations suggest 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 in 4,500-ppm male is not known.

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) that induced urinary bladder calculi formation (43/50; 86%) and transitional cell tumors (31/50; 62%) (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 was reported following as little as 4–8 weeks of dietary exposure to 5,000 ppm biphenyl (Shibata et al., 1989b).

A mode of action involving calculi formation, ulcerations or inflammation, subsequent hyperplasia, and urinary bladder tumor induction has been proposed for other chemicals, including melamine, uracil, and the sodium salt of 2-hydroxybiphenyl, that induce urinary bladder tumors in rodents (Capen et al., 1999; IARC, 1999a, b; Cohen, 1998, 1995). These findings provide further evidence that calculi formation and subsequent 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. 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.

Dose-response concordance. Dose-response relationships for urinary bladder calculi formation, transitional cell hyperplasia, and transitional cell tumor development show concordance in the Umeda et al. (2002) rat bioassay. 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 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 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.*** The available data suggest there may be some ability of biphenyl or its metabolites to induce genetic damage. Genotoxicity testing of 2-hydroxybiphenyl, which is associated with the development of urinary bladder tumors in male rats, provides mixed results. The induction of genotoxic effects by

2-hydroxybiphenyl in the urinary bladder epithelium leading to tumor initiation is proposed to occur via redox cycling between 2,5-dihydroxybiphenyl and phenylbenzoquinone generating reactive oxygen species resulting in oxidative DNA damage (Balakrishnan et al., 2002; Pathak and Roy, 1993; Morimoto et al., 1989). However, no DNA adducts or DNA binding in urinary bladder epithelial 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. 2-Hydroxybiphenyl is a minor urinary metabolite of biphenyl, constituting only a small fraction (0.1–1.0%, Meyer and Scheline, 1976) of the metabolites produced. The metabolite of 2-hydroxybiphenyl responsible for the redox cycling, 2,5-dihydroxybiphenyl, was generally not detected (or detected in trace amounts) in the urine of biphenyl-exposed rats (Meyer and Scheline, 1976). Overall, key mutational events consistent with a mutagenic mode of action for urinary bladder tumors (e.g., mutations in urinary bladder epithelial tissue leading to initiation of tumor cells) are not supported by the available data. Support for a proposed mutagenic mode of action caused by oxidative DNA damage would come from studies showing, for example, formation of 2,5-dihydroxybiphenyl and phenylbenzo-quinone 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.

Relevance of the hypothesized mode of action to humans. The proposed mode of action is expected to be relevant to humans at exposure levels sufficient to cause urinary bladder calculi in humans because 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). 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). In addition, sulphate conjugation of hydroxylated biphenyl metabolites has been demonstrated in human tissues (see Section 3.3), suggesting that humans have the potential to develop calculi.

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. Another physiological factor potentially contributing to reduced susceptibility of humans is the difference in posture between rodents and humans. 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). 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. Increased risks for bladder carcinoma in humans have been associated with cigarette smoking, occupational exposure to polycyclic aromatic hydrocarbons, exposure to *Shistosoma haematobium* that causes urinary tract inflammation, and a history for urinary tract infections in general (Pelucchi et al., 2006; Capen et al., 1999). As such, people with these types of exposure or history may be susceptible to urinary bladder irritation leading to bladder cancer, but evidence supporting this inference is lacking. People with kidney failure, kidney tubular acidosis, urinary tract infection, and vomitting are found to have alkaline urine (Israni and Kasiske, 2011), and could therefore be susceptible to biphenyl-induced calculi formation. 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. Specific evidence supporting these potential susceptibilities is lacking.

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. Liver tumor incidences in male mice showed a statistically significant decrease with increasing dose; the incidences of adenomas and carcinomas in all dose groups were within the range of historical controls for this laboratory. Earlier studies found no liver carcinogenic response in $B6C3F_1$ or $B6AKF_1$ mice exposed to 517 ppm biphenyl in the diet for 18 months (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., 2004a; Sunouchi et al., 1999). Thus, an evaluation of a mode of action involving PPARs 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. Peroxisome proliferators (PPARα agonists) are a structurally diverse group of non- or weakly mutagenic chemicals that activate the PPARs and induce peroxisome proliferation as well as a suite of responses including the induction of tumors in rats and mice. A mode of action for PPARα agonists involving the following key events has been proposed: 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 (Klaunig et al., 2003).

Peroxisome proliferation was once thought to be the sole mode of action for hepatocarcinogenesis induced by PPARα agonists; however, new information in PPARα–null mice (Ito et al., 2007) and in transgenic mouse strains (Yang et al., 2007) have shown that peroxisome proliferation may be neither required nor adequate for hepatocarcinogenicity, and many molecular pathways in different cell types in the liver may contribute to liver cancer development (Guyton et al., 2009). Nonetheless, the remainder of this section considers the extent to which the available experimental data provide support for biphenyl as a PPARα agonist.

4.7.3.2.2. ***Experimental support for the hypothesized mode of action for liver tumors in female mice.*** Data for a possible association between biphenyl-induced proliferation of peroxisomes and liver tumors is limited to findings in BDF_1 mice exposed to biphenyl in the diet for 13 weeks (Umeda et al., 2004a). Identification of peroxisomes was based on light microscopy, with electron microscopic confirmationy performed for liver tissue samples from two control group and two high-dose (16,000 ppm) female mice; no specific staining for peroxisome (e.g., using 3,3'-diaminobenzidene) was performed. Umeda et al. (2004a) reported hepatocellular peroxisome proliferation in the livers of female BDF_1 mice exposed to biphenyl in diet for 13 weeks, but not in male mice. In female mice, evidence of peroxisome proliferation was limited to the 16,000-ppm dose group; no peroxisome proliferation was induced in female mice fed biphenyl at dietary concentrations of 500, 2,000, 4,000, 8,000, or 10,000 ppm. Importantly, Umeda et al. (2004a) did not observe peroxisome proliferation at concentrations (2,000 and 6,000 ppm) that produced statistically significantly increased incidences of liver tumors in the 2-year bioassay in female BDF_1 mice (Umeda et al., 2005). Although peroxisome proliferation was examined in female mice exposed to biphenyl for only 13 weeks (Umeda et al., 2004a), whereas liver tumors were observed after 2 years of exposure (Umeda et al., 2005), a 13-week exposure to biphenyl should have been sufficient to demonstrate induction of peroxisome proliferation. Other studies of PPARα agonists suggest that peroxisome proliferation in the mouse liver (as confirmed by electron microscopy) could occur as early as 10–14 days after treament (Nakajima et al., 2000; Deangelo et al., 1989; Elcombe et al., 1985).

As reported in an abstract only, activities of two enzymes associated with PPARα activation—potassium cyanide-insensitive palmitoyl CoA oxidase (PCO) in liver homogenate and lauric acid 12-hydroxylation in liver microsomes—were significantly increased (up to 1.9- and 3.8-fold, respectively) in female BDF_1 mice given oral doses up to 5.2 mmol/kg-day biphenyl (800 mg/kg-day) for 3 days (Sunouchi et al., 1999). Because PCO activity can vary greatly in both baseline measure and response to chemical exposure, it is not necessarily a consistent indicator of peroxisome proliferation (Laughter et al., 2004; Parrish et al., 1996; Goldsworthy and Popp, 1987; Melnick et al., 1987).

In summary, the available data are not adequate to demonstrate that biphenyl acts as a PPARα agonist or that PPARα agonism is involved in the mode of action for biphenyl-induced liver tumors. In particular, the biphenyl dose associated with peroxisome proliferation in female BDF_1 mice as reported by Umeda et al. (2004a) is not concordant with doses associated with liver tumor induction in Umeda et al. (2005).

4.7.3.2.3. ***Other possible modes of action for liver tumors in mice.*** As discussed in Section 4.5.2, the available data suggest there may be some ability of biphenyl to induce genetic damage. 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.

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 6,000 ppm biphenyl in the diet for 2 years is not supported by the experimental data. This is based on the limited investigation of biphenyl as a PPARα agonist and, in the one available subchronic study, the lack of concordance between dose-response relationships for biphenyl-induced liver tumors and proliferation of hepatocellular peroxisomes in female mice. 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.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No 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 that 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).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

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. It can be derived from a NOAEL, LOAEL, or the 95 percent lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

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

Human studies are preferred over animal studies when quantitative measures of exposure are reported and the reported effects are determined to be associated with exposure (U.S. EPA, 2002); however, no information was located regarding possible associations between oral exposure to biphenyl and health outcomes in humans. In experimental animals, kidney, urinary bladder, liver, and developmental toxicities and decreased body weight were identified as the major effects of biphenyl exposure by the oral route (see Section 4.6.1).

Studies that reported these effects were evaluated using general study quality considerations described in EPA guidance (U.S. EPA, 2002, 1994b). Among the chronic studies that observed effects on the kidney, urinary bladder, and liver and on body weight, the studies by Umeda et al. (2002) in the rat and Umeda et al. (2005) in the mouse were selected as candidate principal studies for dose-response analysis. These were well-conducted studies performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guidelines and Good Laboratory Practice (GLP). Both studies used three biphenyl dose groups plus a control, 50 animals/sex/group, and comprehensive measurement of endpoints. Other chronic studies that evaluated noncancer endpoints (Shiraiwa et al., 1989; Ambrose et al., 1960; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953) reported effects on the kidney and liver, but the Umeda et al. (2005) and Umeda et al. (2002) studies were more comprehensive in the outcomes evaluated and used larger group sizes, supporting the selection of these studies as candidate principal studies.

Other subchronic and chronic studies were less informative as evaluations of the noncancer toxicity of biphenyl and were judged less suitable as candidate principal studies. Endpoints evaluated by Shiraiwa et al. (1989) were limited to body weight, kidney weight, and urinary calculi formation. The studies by Ambrose et al. (1960), Pecchiai and Saffiotti (1957), and Dow Chemical Co (1953) were conducted before the implementation of GLPs and used smaller numbers of animals (8–15/sex/group), which reduced the power of the studies to identify

treatment-related effects. Neither Ambrose et al. (1960) nor Pecchiai and Saffiotti (1957) identified the strain of rat used. The Dow Chemical Co (1953) study was compromised by an outbreak of pneumonia, causing death of all of the control animals. Other chronic studies in mice (Imai et al., 1983; NCI, 1968) reported tumor data only.

Regarding kidney toxicity, the study by Umeda et al. (2002) showed the most sensitive, dose-related measures of kidney effects in the F344 rat to be histopathological changes: renal pelvis transitional cell nodular and simple hyperplasia (males and females), renal pelvis mineralization (males and females), hemosiderin deposits (females only), and papillary mineralization (males and females). These endpoints were selected as candidate critical effects (see Table 5-1). Increased incidences of other histopathologic changes in the kidney (including renal pelvis desquamation in male rats, renal pelvis calculi in male rats, mineralization of the cortico-medullary junction in male rats, papillary necrosis in male and female rats, and infarct in female rats) were observed in high-dose animals only, supporting a continuum of kidney effects increasing in severity with higher exposure that could not be evaluated more comprehensively without individual joint incidence data. While the latter endpoints were not selected for dose-response analysis (see Table 4-5), they were taken into account qualitatively in interpreting the results. In the male and female mouse (Umeda et al., 2005), the most sensitive measures of kidney toxicity were a dose-related increase in the incidence of mineralization in inner stripe of the outer medulla of the kidney and increased urine BUN levels (see Tables 4-7 and 4-8). These endpoints were selected as candidate critical effects.

Table 5-1. Datasets employed in the dose-response 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**
Calculated dose (mg/kg-d)	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Effect								
Renal pelvis								
Nodular transitional cell hyperplasia	0	1	1	21	0	0	1	12
Simple transitional cell hyperplasia	6	8	5	19	3	5	12	25
Mineralization	9	6	10	18	12	12	18	27
Other kidney effects								
Hemosiderin deposit[a]	0	0	0	0	4	8	22	25
Papillary mineralization	9	9	14	23	2	6	3	12
Bladder								
Combined transitional cell hyperplasia[b]	0	0	0	45	1	0	1	10

[a]Male data for incidences of hemosiderin deposits not selected for quantitative analysis.
[b]Female data for incidences of combined transitional cell hyperplasia not selected for quantitative analysis.

Source: Umeda et al. (2002).

Evidence of urinary bladder toxicity is limited to the rat. Umeda et al. (2002) reported histopathologic changes of the bladder in high-dose F344 rats only, with incidences of lesions higher in males than females (see Table 4-4). Histopathological examination showed that the highest incidence of bladder lesions was for transitional cell hyperplasia (simple, nodular, and papillary combined) in male rats; this histopathologic finding was selected as a candidate critical effect. Because the response was more robust in males than that in females, dose-response data for this endpoint in female rats were not modeled.

Liver toxicity associated with biphenyl exposure has been observed primarily in the mouse. Increases in serum liver enzymes (i.e., AST, ALT, LDH, and AP) in female BDF_1 mice observed by Umeda et al. (2005) (see Table 4-7) were the most sensitive measures of biphenyl-related liver toxicity and were selected as candidate critical effects. In general, liver enzyme levels in the male mouse did not show treatment-related changes and were not considered for dose-response analysis.

In the 2-year studies by Umeda et al. (2005) and Umeda et al. (2002), body weights at terminal sacrifice were approximately 20% lower in high-dose F344 rats (males—378 mg/kg-day; females—438 mg/kg-day) than controls and approximately 25–31% lower in high-dose BDF_1 mice (males—1,050 mg/kg-day; females—1,420 mg/kg-day) compared to control. In rats, depression of body weight gain throughout the majority of the study was apparent in high-dose group male and female animals only, whereas biphenyl-related effects on body weight gain in

mice were observed to some extent in all dose groups. Therefore, body weight relative to the control at terminal sacrifice in mice from Umeda et al. (2005) was selected as a candidate critical effect.

In the only developmental toxicity study of biphenyl (Khera et al., 1979), the incidence of fetuses with missing or unossified sternebrae showed an increasing trend with dose that was judged to be biologically significant below the exposure level associated with maternal toxicity. Therefore Khera et al. (1979) was selected as a candidate principal study and incidence of missing or unossified sternebrae in fetuses was selected as a candidate critical effect.

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

No biologically-based dose-response models are available for biphenyl. In this situation, EPA evaluates a range of empirical dose-response models thought to be consistent with underlying biological processes to model the dose-response relationship in the range of the observed data. Consistent with this approach, all standard models available as part of EPA's Benchmark Dose Software (BMDS, version 2.1.2) were evaluated.

Datasets modeled included selected nonneoplastic lesions in the urinary system of F344 rats exposed to biphenyl in the diet for 2 years (Umeda et al., 2002; see Table 5-1); mineralization in the kidney, clinical chemistry parameters, and body weight of BDF_1 mice exposed to biphenyl in the diet for 2 years (Umeda et al., 2005; see Table 5-2); and fetuses with missing or unossified sternebrae from Wistar rat dams administered biphenyl by gavage on GDs 6–15 (Khera et al., 1979; see Table 5-3).

Table 5-2. Datasets employed in dose-response 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**
Kidney histopathology	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	22.9 ± 2.7
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	32.4 ± 3.6
Females				
Dose (mg/kg-d)	**0**	**134**	**414**	**1,420**
Kidney histopathology	n = 50	n = 50	n = 50	n = 49
Mineralization inner stripe-outer medulla	3	5	12	26
Clinical chemistry parameter	n = 28	n = 20	n = 22	n = 31
AST (IU/L)	75 ± 27	120 ± 110	211 ± 373	325 ± 448
ALT (IU/L)	32 ± 18	56 ± 46	134 ± 231	206 ± 280
AP (IU/L)	242 ± 90	256 ± 121	428 ± 499	556 ± 228
LDH (IU/L)	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161
BUN (mg/dL)	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7
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	25.5 ± 3.0

Source: Umeda et al. (2005).

Table 5-3. Dataset for dose-response modeling of incidence of fetuses with missing or unossified sternebrae, from Wistar rat dams administered biphenyl by gavage on GDs 6–15

Effect	Dose (mg/kg-d)			
	0	**125**	**250**	**500**
Fetuses with missing or unossified sternebrae[a]/animals examined (number of litters examined)	4/176 (16)	3/236 (20)	4/213 (18)	16/199 (18)

[a]Data from the 1000 mg/kg-day dose group were not included because of frank maternal toxicity.

Source: Khera et al. (1979).

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), benchmark responses (BMRs) characterizing minimally biologically significant responses for each endpoint were identified where possible. BMDs and BMDLs for body weight decrease were 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 (e.g., in determining maximum tolerated doses). For serum enzyme activities (AST, ALT, AP, LDH), BMDs and BMDLs were calculated for a BMR of 100% increase from the control (i.e., equivalent to a twofold increase, or 1 RD; denoted 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 to indicate liver injury in experimental animals.

For reproductive and developmental studies with nested designs, a BMR of 5% extra risk in individual offspring has been used analogously to 10% extra risk in adults to reflect greater susceptibility during this critical window of development. To be able to use nested models, the numbers of affected and total fetuses within each litter are required, which were not included in the Khera et al. (1979) study report. An approach that uses dichotomous models to approximate the result of nested models was used, as follows. First, note that although the BMD corresponding to a particular fetal risk (e.g., 5% extra risk) can be estimated correctly using the incidence of affected fetuses among the total number of live fetuses (Williams and Ryan, 1997; Haseman and Kupper, 1979; Haseman and Hogan, 1975), it is the BMDL that cannot be estimated correctly without the numbers of both affected and total fetuses within each litter to calculate the variance. The correct variance estimate lies between the variance with total litters as sample size and the variance with total fetuses as sample size (Rao and Scott, 1992). Consequently, the dichotomous models in BMDS were fit to the proportions of fetuses affected in two separate analyses—one with the number of litters in each dose group as sample sizes, and one with the total number of fetuses in each dose group as sample sizes (Table 5-3). These two sets of modeling results bracket the BMDL that would result from nested modeling.

In the absence of information regarding what level of change is considered biologically significant, the BMD and BMDL were estimated using a BMR of 10% extra risk for dichotomous data (e.g., hyperplasia), or a BMR of 1 SD from the control mean for continuous data (e.g., BUN). For all endpoints, these latter BMRs (a BMR of 1 SD for continuous data or 10% extra risk for dichotomous data) were also used to facilitate a consistent basis of comparison across endpoints, studies, and assessments.

In general, adequate model fit was judged by the χ^2 goodness-of-fit p-value ($p \geq 0.1$), visual inspection of the fit of the dose-response curve to the data points, scaled residuals, and fit in the low-dose region and in the vicinity of the BMR. For continuous data, the assumption of constant variance in the responses across each set of dose groups was tested. If the assumption was met ($p \geq 0.1$), the fit of continuous models to the mean was evaluated while assuming

constant variance; if not, all models were evaluated while applying the power model integrated into BMDS to account for nonhomogeneous variance.

If standard models failed to provide adequate fit to the data, modifications of these standard models (i.e., parameter restriction adjustments) or use of alternative models were considered in an effort to achieve adequate fit. Then if adequate fit could not be achieved, the highest dose was dropped and the entire modeling procedure was repeated. If no adequate fit could be achieved after dropping the highest dose, then the dataset was regarded as not amenable for BMD modeling.

Among all of the models providing adequate fit to a dataset, the model with the lowest Akaike's Information Criterion (AIC) was chosen as the best-fitting model when the difference between the BMDLs estimated from a set of models was less than threefold. Otherwise, the model with the lowest BMDL was selected as the best-fitting model for a dataset (U.S. EPA, 2012). If datasets could be adequately modeled, then the BMDLs from the selected models were used as candidate points of departure (PODs). If not, NOAEL or LOAEL values were considered as candidate PODs.

Summary modeling results are presented in Table 5-4 and Figure 5-1; more detailed modeling results are presented in Appendix D (Tables D-4 through D-24 and respective model output files). The BMDs and BMDLs shown in Table 5-4 and Figure 5-1 are those from the best-fitting models for each endpoint. BMD and BMDL for serum AST levels in female mice were derived after dropping the data from the highest dose groups.

Table 5-4. Summary of candidate PODs 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	Logistic	10%	234	192	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	NA				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	NA			
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	NA				Power	1 RD	190[a]	122[a]
ALT	NA				No adequate fit[a]	1 RD	–	–
LDH	NA				No adequate fit[a]	1 RD	–	–
AP	NA				No adequate fit[a]	1 RD	–	–
BUN	No adequate fit[a]	1 SD	–	–	No adequate fit[a]	1 SD	–	–
Body weight								
Terminal body weight.	No adequate fit[a]	0.1 RD	–	–	Linear	0.1 RD	583	511
Wistar rats (Khera et al., 1979); biphenyl by gavage to dams on GDs 6–15								
Fetuses with missing or unossified sternebrae, sample size = number of litters in each dose group					Log-logistic[b]	5%	477	173
Fetuses with missing or unossified sternebrae, sample size = number of fetuses in each dose group					Multistage 3-degree[b]	5%	460	382

[a]"No adequate fit" indicates that none of the models in BMDS provided an adequate fit to the data. Where BMD/BMDL values could not be derived, NOAELs were used as the POD. NOAELs for male mice: BUN–97 mg/kg-day; body weight–291 mg/kg-day. NOAELs for female mice: AP–414 mg/kg-day; ALT, LDH, and BUN–134 mg/kg-day.
[b]Data from the 1,000 mg/kg-day dose group were not included because of frank maternal toxicity.

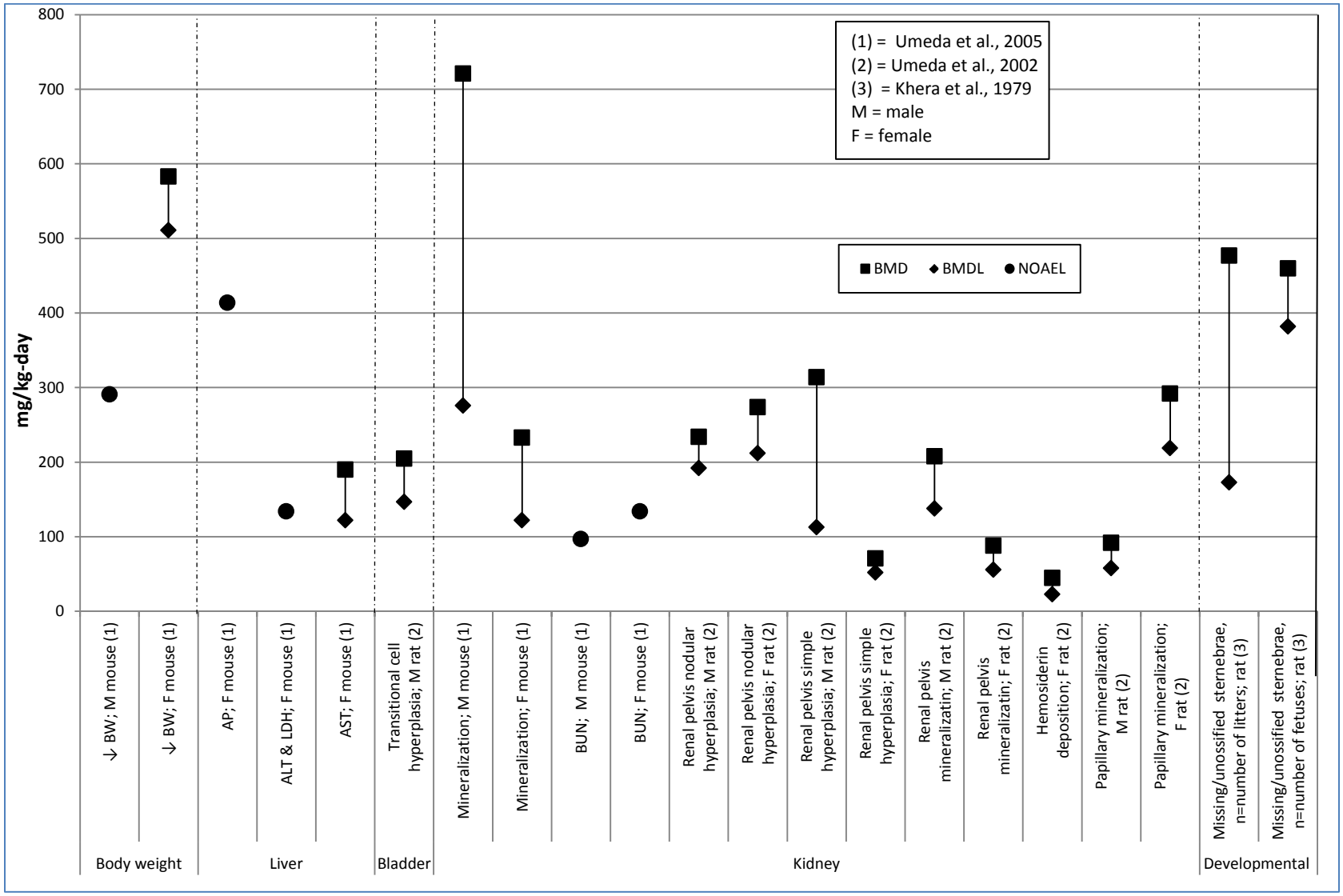


Figure 5-1. Candidate PODs for selected noncancer effects in rats and mice from repeated oral exposure to biphenyl.

Selection of the critical effect. Based on the results of dose-response modeling presented in Table 5-4 and Figure 5-1, the kidney of rats exposed to biphenyl in the diet for 2 years appears to be the most sensitive target of biphenyl toxicity in both male and female F344 rats, with the lowest BMD_{10} values obtained. These results ranged from 45–92 mg/kg-day, corresponding to renal pelvis simple transitional cell hyperplasia and mineralization (females), renal papillary mineralization (males), and hemosiderin deposition (females). As discussed in Section 4.6.1, in the kidney medulla, papillary mineralization falls on a continuum of effects progressing (at higher doses) to papillary necrosis, and is consistent with a functional change in the kidney. Papillary mineralization was a more sensitive endpoint among male rats than female rats, with BMD_{10} values of 92 and 292 mg/kg-day, respectively. At the same time, the female rats showed more sensitive results than the males for renal pelvis simple transitional cell hyperplasia and and mineralization, with BMD_{10} values of 71–88 mg/kg-day, compared with 208–314 mg/kg-day in the males. Although the BMD_{10} for hemosiderin deposits in the female rat was lower (by about twofold) than the value associated with papillary mineralization, the biological relevance of hemosiderin deposits as reported in Umeda et al. (2002) is unclear (see Section 4.6.1). Papillary mineralization in male rats was selected as the critical effect and the basis for derivation of the RfD because it was judged to be the more serious outcome in this range of BMD_{10} values, given its likely progression to necrosis at higher exposures. Similar results for the other kidney histopathology outcomes support this selection.

Derivation of human equivalent doses (HEDs). HEDs for oral exposures were derived from the PODs estimated from the laboratory animal data, as described in EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011). In this guidance, EPA advocates a hierarchy of approaches for deriving HEDs from data in laboratory animals, with the preferred approach being physiologically-based toxicokinetic modeling. Other approaches can include using chemical-specific information in the absence of a complete physiologically-based toxicokinetic model. Since a validated human PBPK model for biphenyl for extrapolating doses from animals to humans is not available, in lieu of either chemical-specific models or data to inform the derivation of human equivalent oral exposures, a body weight scaling to the ¾ power (i.e., $BW^{3/4}$) approach was applied to extrapolate toxicologically equivalent doses of orally administered biphenyl from adult laboratory animals to adult humans for the purpose of deriving an oral RfD. Consistent with EPA guidance (U.S. EPA, 2011), the PODs estimated based on effects in adult animals was converted to HEDs employing a standard dosimetric adjustment factor (DAF) derived as follows:

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

where BW_a = animal body weight and BW_h = human body weight

Using a BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF for rats was 0.24, respectively. Applying this DAF to the POD identified for effects in adult rats yields a POD_{HED} as follows:

POD_{HED} = laboratory animal dose (mg/kg-day) × DAF

The POD for deriving the RfD for biphenyl (i.e., the $BMDL_{10}$ for papillary mineralization in male rats) was converted to a POD_{HED} as follows:

POD_{HED} = $BMDL_{10}$ (mg/kg-day) × DAF
= 58 mg/kg-day × 0.24
= 13.9 mg/kg-day

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

Consideration of available dose-reponse data led to the selection of the 2-year bioassay of biphenyl in the F344 rat (Umeda et al., 2002) and papillary mineralization as the principal study and critical effect, respectively, for RfD derivation. The UFs, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), addressed five areas of uncertainty resulting in a composite UF of 30. This composite UF was applied to the selected POD to derive an RfD.

- An interspecies uncertainty factor of 3 (UF = $10^{1/2}$ = 3.16, rounded to 3) is applied because $BW^{3/4}$ scaling is being used to extrapolate oral doses from laboratory animals to humans. Although $BW^{3/4}$ scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's $BW^{3/4}$ guidance (U.S. EPA, 2011) recommends use of an uncertainty factor of 3.

- 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 subchronic to chronic extrapolation in this assessment because the candidate principal study was chronic in duration.

- An UF of 1 was applied for LOAEL to NOAEL extrapolation 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% increased incidence of papillary mineralization in the rat kidney was selected under the 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., 2004a); 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. Seppalainen and Hakkinen (1975) reported abnormal EEG and ENMG findings and increases in clinical signs 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 an increased prevalence of Parkinson's disease in a Swedish factory manufacturing biphenyl-impregnated paper where exposures were likely to have exceeded the TLV of 1.3 mg/m^3. The evidence of an association between biphenyl exposure and increased prevalence of Parkinson's disease was not confirmed by the earlier study by Seppalainen and Hakkinen (1975), despite workplace concentrations that appeared to be considerably higher than those in the plant investigated by Wastensson et al. (2006). Wastensson et al. (2006) acknowledged that chance is an alternative explanation for the cases identified in the Swedish factory workers. Animal studies did not include examination of sensitive measures of neurotoxicity; however, the 2-year oral bioassays in rats and mice (Umeda et al., 2005; Umeda et al., 2002) did include daily observations for clinical signs and histopathological examination of nervous system tissues. No nervous system effects were reported, suggesting that the nervous system is not a sensitive target of oral biphenyl toxicity. Overall, the findings from studies of occupational (predominantly inhalation) exposure to biphenyl introduce some uncertainties in the characterization of biphenyl hazard by ingestion, but were not considered a data gap sufficient to warrant a database UF.

The RfD for biphenyl was calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{POD}_{\text{HED}} \div \text{UF} \\ &= 13.9 \text{ mg/kg-day} \div 30 \\ &= 0.46 \text{ mg/kg-day, or } 0.5 \text{ mg/kg-day rounded to one significant figure} \end{aligned}$$

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)

The RfC (expressed in units of mg/m^3) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95% lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

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

Human studies are preferred over animal studies when quantitative measures of exposure are reported and the reported effects are determined to be associated with exposure (U.S. EPA, 2002). The available human data for biphenyl are limited to two occupational epidemiology studies and a case report of workers engaged in the production of biphenyl-impregnated fruit wrapping paper (Carella and Bettolo, 1994; Seppalainen and Hakkinen, 1975; Häkkinen et al., 1973; Häkkinen et al., 1971). 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. These studies were evaluated using general study quality considerations described in EPA guidance (U.S. EPA, 2002, 1994b). In three separate studies that included repeated inhalation exposure of rabbits, rats, and mice to air containing 300, 40, or 5 mg/m^3 biphenyl, respectively, 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). Limitations in study design, including lack of control animals and use of a single exposure level, as well as poorly reported study details, preclude the use of these studies 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). Study limitations and lack of supporting data preclude the use 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. 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 (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 PBPK model. Although an RfC cannot be derived, it should be noted that the available inhalation data provide 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

This section provides 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 13.9 mg/kg-day (see Section 5.1). Uncertainty 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 endpoint selected for derivation of the RfD is increased incidence of kidney papillary mineralization in F344 rats as reported by Umeda et al. (2002). The fact that kidney effects have been consistently associated with biphenyl exposure in multiple oral studies in male and female rats (Umeda et al., 2002; Shiraiwa et al., 1989; Ambrose et al., 1960; Pecchiai and Saffiotti, 1957; Dow Chemical Co, 1953) and in one study in male and female mice (Umeda et al., 2005) provides a measure of confidence that the kidney is a target of biphenyl toxicity. Kidney effects have not been reported in populations exposed to biphenyl in the workplace, however, and there is some degree of uncertainty associated with extrapolation of kidney effects in experimental animals to humans. As discussed in Section 4.7.3.1.4 (in the context of the relevance of rat urinary bladder tumors to humans), physiological factors such as urine pH appear to play a role in the formation of calculi by biphenyl. To the extent that these physiological factors influence the renal response to

biphenyl, the response in humans and rodents to biphenyl could differ. The lack of understanding of physiological factors that influence susceptibility to biphenyl exposure introduces uncertainty in the RfD.

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. Although the selected model (i.e., multistage model) provided the best mathematical fit to the papillary mineralization in the male rat, (as determined by the criteria described in Section 5.1.2), this model does not necessarily have greater biological support over the various other models that were available. Some BMDS models yielded estimates of the POD that were similar to the selected POD), and other models yielded values for the POD approximately twofold higher than the best fitting model.

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 result in effects on the lungs or other systemic targets. The lack of adequate data to derive an RfC represents a significant data gap.

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; Umeda et al., 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

weight of evidence, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk.

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. A review of the available chronic animal bioassays of biphenyl, including strengths and limitations, is provided in Section 4.7.2. Two well-conducted animal bioassays found a statistically significant increasing trend in urinary bladder tumors in male, but not female, F344 rats (Umeda et al., 2002) and a statistically significant increasing trend in liver tumors in female, but a statistically significant decreasing trend in liver tumors in male BDF_1 mice (Umeda et al., 2005). Although decreased, the incidences of male liver tumors remained within the historical control range of this laboratory. Further, similar decreased trends in liver tumors that were associated with decreased body weight gain in $B6C3F_1$ mice, as also occurred with the BDF_1 mice exposed to biphenyl, have been judged not to demonstrate anticarcinogenicity [e.g., Leakey et al. (2003); Haseman and Johnson (1996)]. Although no mechanistic data or methodological differences were identified that could further explain the differing results between sexes, the lack of increased tumor responses in female rats or in male mice does not invalidate the positive findings. Consequently, the tumor data for male rat urinary bladder tumors and female mice liver tumors 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 5-6. 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 5-7. The datasets selected for dose-response analysis include urinary bladder transitional cell papilloma or carcinoma in male F344 rats and liver adenoma or carcinoma in female BDF_1 mice. In both the urinary bladder and liver, benign and malignant tumors were considered together because benign and malignant tumors in both of these organs develop from the same cell lines and benign tumors can progress to carcinomas (U.S. EPA, 2005a; McConnell et al., 1986).

Table 5-6. 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)	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Tumor incidence[a]								
Transitional cell								
Papilloma	0/50	0/50	0/50	10/49*	0/50	0/50	0/50	0/50
Carcinoma	0/50	0/50	0/50	24/49*	0/50	0/50	0/50	0/50
Papilloma or carcinoma	050	0/50	0/50	31/49**	0/50	0/50	0/50	0/50

[a]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.
*Statistically significant (Fisher's exact test, $p < 0.05$) as reported by study authors.
**Statistically significant (Fisher's exact test, $p < 0.05$) as determined by EPA.

Source: Umeda et al. (2002).

Table 5-7. Incidence data for liver tumors in male and female BDF_1 mice fed diets containing biphenyl for 2 years

	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*	10/48*
Carcinoma	8/50	8/49	5/49	4/50	1/48	5/50	7/49*	5/48
Adenoma or carcinoma	16/50	12/49	9/49	7/50	3/48	8/50	16/49*	14/48*

[a]One low-dose and one mid-dose male mice and 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.
*Statistically significant (Fisher's exact test, $p < 0.05$) as reported by study authors.

Source: Umeda et al. (2005).

5.4.3. Dose Adjustments and Extrapolation Method(s)

5.4.3.1. *Liver Tumors in Female Mice*

A scaling approach based on $BW^{3/4}$ was used to extrapolate toxicologically equivalent doses of orally administered dose from laboratory animals to humans. Mouse body weights from Umeda et al. (2005) were estimated from data provided on average daily food consumption and

intake.[7] Scaling factors were calculated as $BW_a^{1/4} / BW_h^{1/4}$ (U.S. EPA, 2005a; U.S. EPA, 1992), where BW_a = average body weight for each dose group of female mice and BW_h = average human body weight (70 kg) (U.S. EPA, 1988). The HED was calculated as: HED = scaling factor × reported dose (Table 5-8).

Table 5-8. 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 = $(\text{average mouse body weight}/70)^{0.25}$.
[c]HED = reported dose × scaling factor.

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. 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 in the female mouse used to derive the oral slope factor are presented in Table 5-9. 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.

[7]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.

Table 5-9. Incidence of liver adenomas or carcinomas 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,*]	14/48[a,*]

[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.
[*]Statistically significant (Fisher's exact test, $p < 0.05$) as reported by study authors.

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[8] 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 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, consistent with EPA guidance (U.S. EPA, 2005a), as a 10% response 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 the same three criteria used for noncancer modeling. If an adequate fit to the data was not achieved with the multistage models, 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 E, Table E-2). The incidence of liver tumors showed a plateau in animals in the two highest dose groups. The lack of a monotonic increase in liver tumor incidence in the high-dose group could not be attributed to higher mortality, as the survival rate in the high-dose group was comparable to controls and the low- and medium-dose groups. 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 E, Table E-2). The $BMD_{10/HED}$ and $BMDL_{10/HED}$ using this latter dataset were 18.7 and 12.2 mg/kg-day, respectively. See Appendix E for more information.

[8]The multistage model is mathematically identical to multistage cancer model.

5.4.3.2. *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). Based on the proposed mode of action, exposure to biphenyl at doses that would not result in calculi formation and subsequent key events would not be associated with bladder tumors. 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.

Bladder calculi, the formation of which is a key event in the mode of action for urinary bladder tumors, were observed in male rats (Umeda et al., 2002) bioassay at a dose of 378 mg/kg-day; the NOAEL for these effects was 110 mg/kg-day. The HED for this NOAEL is 26 mg/kg-day, derived by application of a DAF of 0.24 (see Section 5.1.2 for discussion of the DAF). A candidate RfD for bladder calculi of 0.9 mg/kg-day is derived by applying a composite UF of 30 to this HED (see Section 5.1.3 for discussion of UFs). The RfD of 0.5 mg/kg-day based on papillary mineralization in kidney is approximately twofold below the candidate RfD for bladder calculi of 0.9 mg/kg-day. Based on the proposed mode of action, it is anticipated that exposure to biphenyl at doses that would not result in calculi formation would not be associated with an increased risk of bladder tumors.

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 extra risk (i.e., BMR of 10% extra risk) at the POD by the corresponding BMDL ($0.1/BMDL_{10/HED}$). Using linear extrapolation from the $BMDL_{10/HED}$, the human equivalent oral slope factor of 8.2×10^{-3} $(mg/kg\text{-}day)^{-1}$ (rounded to one significant figure, 8×10^{-3} $(mg/kg\text{-}day)^{-1}$) was derived for liver tumors in female BDF_1 mice (Table 5-10).

Table 5-10. 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	$BMD_{10/HED}$ (mg/kg-d)	$BMDL_{10/HED}$ (mg/kg-d)	Slope factor[a] (risk per [mg/kg-d])
Female mouse liver tumors	18.7	12.2	8×10^{-3}

[a]Human equivalent slope factor = $0.1/BMDL_{10/HED}$; see Appendix E 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 5-11 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 5-11. 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 tumor data sets with mode-of-action information	The Umeda et al. (2005) study was selected.	The bioassay by Umeda et al. (2005) was a well-conducted experiment with four dose groups (including control) and 50 animals/sex/group.
Cross-species scaling	Alternatives could ↑ or ↓ slope factor (e.g., 7-fold ↓ [scaling by body weight] or twofold ↑ [scaling by $BW^{2/3}$] for mouse liver tumor)	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.

Table 5-11. Summary of uncertainties in the biphenyl cancer slope factor

Consideration/ approach	Impact on slope factor	Decision	Justification
Extrapolation procedure for rat urinary bladder tumors	Not applicable; mode of action for male rat bladder tumors does not support low-dose linear extrapolation	Nonlinear extrapolation; the RfD of 0.5 mg/kg-day is based on a POD of 58 mg/kg-day, which is ~twofold lower than the NOAEL for bladder calculi.	Available mode-of-action data for urinary bladder tumors support nonlinearity (i.e., that bladder tumor is a high-dose phenomenon and is closely related to calculi formation in the urinary bladder of male rats). An uncertainty analysis was performed based on the assumption that another mode of action for urinary bladder tumors might be operative; for this analysis, a linear extrapolation approach was used. See text below for further details of this analysis.
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 mode-of-action data do not inform selection of a dose-response model. Linear approach in the 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	Female mouse liver tumor incidence was chosen for dose-response analysis.	Dietary exposure to biphenyl induced increased incidences of liver tumors in female mice, and a decreased trend of liver tumors in male mice. 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 by an unknown extent	Drop highest dose of the liver tumor dataset.	Model options explored with the full liver tumor dataset did not provide adequate fit ($p \geq 0.05$). Dropping the highest dose allowed a better fit to the low-dose region of the data set.
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 limit 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.

Two members of the peer review panel offered the views that the data do not prove that bladder stones are required for carcinogenesis and that an alternative mode of carcinogenic action was not adequately investigated. To explore the situation where the mode of action is unknown, a linear extrapolation approach was performed. A slope factor of 2×10^{-3} $(mg/kg\text{-}day)^{-1}$ was derived from a $BMDL_{10/HED}$ of 41.2 mg/kg-day based on incidence of bladder tumors in male rats and linear low-dose extrapolation from the $BMDL_{10/HED}$ (see Appendix E for BMD modeling documentation). This slope factor is lower than the slope factor derived from mouse liver tumors, indicating that urinary bladder tumors are less likely than liver tumors at a given

exposure under the assumption of low-dose linearity. Because the available data support calculi formation as a key event in the mode of action for male rat urinary bladder tumors, EPA does not consider linear low-dose extrapolation to be supported for this tumor type.

The uncertainties presented in Table 5-11 have a varied impact on risk estimates. Some suggest that 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 mode of action 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 posted to the IRIS database in 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., 1976b; Meyer et al., 1976a). Limited data show that biphenyl can be absorbed by human skin (DuPont, 2005). Data for absorption, distribution, and elimination are not available for inhaled 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 effects were observed.

Chronic oral studies in rats and mice identify the liver and urinary system as principal targets of biphenyl toxicity. In rats exposed to biphenyl in the diet for 2 years, nonneoplastic kidney lesions (including histopathological changes in the renal pelvis and papilla of the medulla) were found at dietary concentrations ≥1,500 ppm (≥128 mg/kg-day). 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 the only available developmental toxicity study for biphenyl, the incidence of fetal skeletal anomalies (mainly missing or unossified sternebrae) showed a significantly increasing trend with exposure to biphenyl on GDs 6–15 (Khera et al., 1979).

Biphenyl effects on reproductive function in rats have been reported at exposure levels higher than those associated with effects on the urinary tract, liver, or developing fetus. 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). In general, the toxicity of inhaled biphenyl is poorly characterized because the available inhalation studies are limited by study methodology and reporting issues.

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 (Umeda et al., 2002), 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 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 high-dose (438 mg/kg-day) female rats were observed at lower incidence and were different in physical appearance and chemical composition; furthermore, there were no urinary bladder tumors in any biphenyl-exposed female rats.

In a 2-year study of BDF_1 mice administered biphenyl in the diet (Umeda et al., 2005), the incidence of liver tumors in female mice was significantly increased at doses ≥414 mg/kg-day. In male mice, liver tumor incidence showed a statistically significant decrease with increasing dose, although the incidences were within the range of historical control data for adenomas or carcinomas in male mice. 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." This cancer descriptor is based on an increase in the incidence of urinary bladder tumors (transitional cell papillomas and carcinomas) in male F344 rats (Umeda et al., 2002) and liver tumors (hepatocellular adenomas and carcinomas) in female BDF_1 mice (Umeda et al., 2005) 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 0.5 mg/kg-day was based on an increased incidence of renal papillary mineralization (Umeda et al., 2002). To derive the RfD, the POD_{HED} was divided by a composite UF of 30 (3 for animal-to-human extrapolation and 10 for human interindividual variability in susceptibility). The interspecies UF was applied to account for residual uncertainty in the extrapolation of toxicokinetic and toxicodynamic processes 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 (Umeda et al., 2002) is high. Umeda et al. (2002) is a well-conducted study performed in accordance with OECD test guidelines and GLPs. Confidence in the database is medium to high. The database is robust in that it includes well-conducted chronic oral exposure studies in the rat and mouse, other supporting repeated dose studies in multiple species, a developmental toxicity study in Wistar rats, and one- and three-generation reproductive toxicity studies in rats. Confidence in the database is reduced because the reproductive toxicity studies come from the older toxicological literature (1953 and 1960) and do not fully evaluate effects of biphenyl exposure on reproductive function as would studies conducted using current study protocols.

6.2.2. Noncancer/Inhalation

No inhalation RfC was derived due to the lack of inhalation 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, tracheal hyperplasia, and 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. The HED for this NOAEL is 26 mg/kg-day, derived by application of a DAF of 0.24 (see Section 5.1.2 for discussion of the DAF). A candidate RfD for bladder calculi of 0.9 mg/kg-day is derived by applying a composite UF of 30 (3 for residual interspecies differences, 10 for intraspecies variability in susceptibility) to this HED. The RfD of 0.5 mg/kg-day based on papillary mineralization in kidney is approximately twofold below the candidate RfD for bladder calculi of 0.9 mg/kg-day. Based on the proposed mode of action, it is anticipated that exposure to biphenyl at doses that would not result in calculi formation would not be associated with an increased risk of bladder tumors.

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

The Toxicological Review of Biphenyl, dated September 2011, has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). An external peer-review workshop was held on April 3, 2012. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments follow. In many cases, the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA also received scientific comments from the public. Public comments are posted to the federal docket at www.regulation.gov; search for docket ID No. EPA-HQ-ORD-2011-0739.[9] A summary of these public comments and EPA's responses are included in a separate section of this appendix.

I. External Peer Review Comments

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

General Comments

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer health effects of biphenyl?

Comments: To varying degrees, all of the reviewers commented that the draft was well written, logical, clear, and generally well done. Four reviewers commented that the document was not concise or that there was some redundancy in the information presented; two reviewers, on the other hand, specially stated that the document was concise. Several reviewers suggested that clear and concise conclusions at the end of each section (in particular, the Toxicokinetics section) or introductory paragraphs at the beginning of major sections would be helpful. One reviewer identified several statistical issues (e.g., failure to identify a finding as statistically significantly different from the control in summary tables and questions about the application of certain statistical tests).

[9] Public comments on the draft biphenyl Toxicological Review posted to www.regulations.gov can be found at the following URL: http://www.regulations.gov/#!docketDetail;D=EPA-HQ-ORD-2011-0739.

Response: The Toxicological Review was revised throughout to reduce redundancy, and information of lesser relevance throughout the document was removed to the extent practicable. Summaries of biphenyl toxicokinetics and human health effects information were added to the beginning of Sections 3 and 4.1. A summary of animal studies was already included in Section 4.2. Section 4.6 was revised to provide a more comprehensive review and synthesis of biphenyl health effects information. Statistical errors and omissions were corrected.

Comments: One reviewer recommended further discussion of the evaluation of older studies of cancer and noncancer endpoints, including more details on the strengths and weaknesses of these studies, and more explanation as to how each study contributed to the final decision making.

Response: Section 4.6.1, Synthesis of Major Noncancer Effects, concerning the noncancer effects of biphenyl and Sections 4.7.1, Summary of Overall Weight of Evidence, and 4.7.2, Synthesis of Human, Animal, and Other Supporting Evidence, concerning the carcinogenicity of biphenyl were revised, as appropriate, to more explicitly take into consideration study quality in identifying the hazards associated with biphenyl exposure. Section 5.1.1, Choice of Candidate Principal Studies and Candidate Critical Effects – With Rationale and Justification, was revised to include a more explicit evaluation of the strengths and weakness of major studies and the rationale for choosing studies for dose-response analysis.

Comments: One reviewer recommended that a description of the literature search strategy for locating relevant literature be included.

Response: Documentation of the literature search strategy, including a graphical depiction of the literature search strategy and search outcomes, was added as Appendix B of the Toxicological Review; reference to this appendix was added to Section 1. The search strategy documentation also provides a link to EPA's Health and Environmental Research Online (HERO) database (www.epa.gov/hero) that contains a web page showing the references that were cited in the Toxicological Review as well as those references identified in the literature search that were screened (considered) but not cited.

Comments: One reviewer observed that Section 4, Hazard Identification, was well written, clear, and concise, but offered suggestions for presentation or clarification beyond those provided in response to specific charge questions.

- The reviewer noted that the incidence of reticular cell sarcoma in biphenyl-treated female strain B mice (summarized in Table 4-9) was significantly greater than in controls by Fisher Exact Test ($p < 0.01$), and should be noted in Table 4-9 and briefly discussed in accompanying text and Section 4.7, Evaluation of Carcinogenicity.

- The nonrodent oral studies reported in Section 4.2.1.2.3 are shorter than one-tenth of the lifespan of the animal species and should not be included in the "Chronic toxicity and carcinogenicity studies" section (i.e., 1-year dog and 1-year Rhesus monkey studies). The reviewer recommended that these studies be moved to a separate section or included in the subchronic study section.

- The reviewer stated that the overall weight of evidence for genotoxicity appears more equivocal than negative given the clastogenicity in human lymphocytes, the in vivo findings, and the limited evidence for genotoxicity of metabolites.

- Regarding statements in the mode of action section related to lack of concordance for neurotoxicity between humans and animals, the reviewer observed that the animal studies were not designed to detect the neurotoxicity seen in human studies.

Response: EPA has noted the statistical significance of the increased incidence of reticular cell sarcoma in strain B female mice (NCI, 1968) in Section 4.2.1.2.2 and Table 4-9. Discussion of the biological significance of this tumor finding was added to Sections 4.2.1.2.2 and 4.7.1. EPA agrees that the 1-year dog and monkey studies should not be considered chronic-duration studies. Summaries of these nonrodent oral studies in Section 4.2.1.2.3 were moved to Section 4.2.1.1 (Subchronic Toxicity). Section 4.5.2 (Genotoxicity) and Appendix C were revised to more precisely characterize the available evidence for the genotoxicity of biphenyl and its metabolites. The comment related to evidence for neurotoxicity associated with biphenyl exposure is addressed in responses under Charge Question A.4.

2. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of the noncancer and cancer health effects of biphenyl.

Comments: Seven of the eight reviewers did not identify any additional studies. One reviewer recommended consideration of an issue of the journal *Birth Defects Research* that was devoted to interpreting skeletal malformations and variations (Birth Defects Research, Part B, 80(6), 2007). This reviewer stated that articles in this volume address some of the malformations found in the Khera et al. (1979) study and may directly impact the consideration of using skeletal malformations as the endpoint for calculation of the RfD.

Response: EPA agrees that the recommended journal issue is pertinent for this assessment. Discussion of a particular paper from this issue (Carney and Kimmel, 2007) was added to Section 4.6.1 to support interpretation of fetal skeletal variations as reported by Khera et al. (1979).

A. Oral Reference Dose (RfD) for Biphenyl

The first two charge questions in this portion of the review address the selection of the critical effect and the principal study for developing an RfD. For this database, the critical endpoint used in the draft assessment and another recommended by reviewers were specific to different studies [i.e., skeletal anomalies as reported in a developmental toxicity study by Khera et al. (1979) and renal endpoints as reported in a chronic bioassay in the rat by Umeda et al. (2002)]. As such, preference for one endpoint also determines the choice of study. For this reason, the comments and responses to the following two related charge questions were merged.

1. A developmental toxicity study of biphenyl in Wistar rats (Khera et al., 1979) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfD, please identify this study and provide scientific support for this choice.

2. A developmental effect in Wistar rats (i.e., fetal skeletal anomalies) was concluded by EPA to be an adverse effect and was selected as the critical effect for the derivation of the RfD. Please comment on whether the selection of this critical effect and its characterization is scientifically supported and clearly described. If a different endpoint is recommended as the critical effect for deriving the RfD, please identify this effect and provide scientific support for this choice.

Comments: Several peer reviewers raised concerns about the selection of fetal skeletal anomalies in Khera et al. (1979) as the critical effect, and proposed as an alternative critical effect renal lesions as reported in the 2-year rat bioassay of biphenyl by Umeda et al. (2002). More specifically, three reviewers commented that justification for the selection of fetal skeletal anomalies as the critical effect needed to be expanded, noting that consideration should be given to maternal toxicity and whether delayed ossification and extra ribs are adverse effects. One of these reviewers commented that it is difficult to determine the appropriateness of selecting Khera et al. (1979) as the principal study without more details on the fetal anomalies—details that were not provided in the published study. Two reviewers did not support selection of fetal skeletal anomalies as the critical effect. One of these two reviewers did not consider the skeletal anomalies to be adverse findings in the absence of other malformations, and concluded that the anomalies could be attributed to maternal toxicity. Two reviewers expressed concern about the quality of the developmental study conducted approximately 35 years ago. On the other hand, three reviewers considered the selection of fetal skeletal anomalies as reported by Khera et al. (1979) either to be appropriate, consistent with EPA guidelines, or clearly described.

Five reviewers, including one who considered the selection of fetal skeletal anomalies a reasonable choice and consistent with EPA guidelines, identified renal lesions as reported in the 2-year rat bioassay by Umeda et al. (2002) as an alternative or more scientifically defensible critical effect. One reviewer specifically recommended hemosiderin deposition in the kidney (Umeda et al., 2002) as an alternative critical effect, whereas another reviewer considered hemosiderin to be a nonspecific effect that "usually is meaningless to humans." The latter reviewer recommended simple hyperplasia of the kidney, renal pelvis mineralization, or papillary mineralization as more scientifically defensible as the critical effect.

Response: EPA agrees that the Khera et al. (1979) study may have differed from more current study designs, but is unaware of any particular study quality issues due to the age of the study that would decrease confidence in its conduct or reported results. The study design used a typical number of rats (18−20 dams/dose group), used four dose groups after consideration of the results of a range-finding study, and evaluated skeletal and visceral anomalies using standard methods. As it is the only developmental toxicity study of biphenyl available, there is no corroboration of the findings. Without any indication that the study was designed or conducted in an inappropriate manner, however, these findings have a place in the hazard evaluation of biphenyl.

EPA agrees that the uncertainties in the interpretation of fetal skeletal anomalies, including maternal toxicity and adversity of the anomalies, were not adequately weighed in selecting this endpoint as the critical effect for the RfD. Discussion of the Khera et al. (1979) study was revised to more clearly present the following points that influenced interpretation of the study findings. Maternal toxicity was observed in the highest dose group (1,000 mg/kg-day), but not at ≤500 mg/kg-day. Skeletal anomalies were found at ≤500 mg/kg-day, and thus, cannot be attributed to maternal toxicity. Among the anomalies listed, missing or unossified sternebrae was the only endpoint elevated with increasing dose at doses lower than 1,000 mg/kg-day. Consistent with reviewers' advice and the more recent publications they recommended (e.g., Carney and Kimmel, 2007), anomalies with biological significance were limited to missing or unossified sternebrae.

The Khera et al. (1979) study was retained as a candidate principal study. In light of the issues raised by the reviewers, however, EPA clarified the interpretation of the anomalies in this study in Sections 4.3.1 and 4.6.1. In addition, EPA listed the anomalies observed in "anomalous litters"—wavy ribs, extra ribs, missing or unossified sternebrae, or delayed ossification of the calvarium—and included the respective incidences of fetuses in each dose group (see Section 4.3.1). The incidence of missing or unossified sternebrae and the number of litters examined were repeated in the dose-response section.

Consistent with peer reviewer recommendations, the robust toxicity studies in rats and mice by Umeda et al. (2005) and Umeda et al. (2002) were also considered as candidate principal

studies, with the rationale clarified in Section 5.1.1. Also consistent with peer reviewer recommendations, renal lesions, and in particular renal papillary mineralization in male rats, was selected as the critical effect. Sections 4.6.1 and 5.1.2 were revised to better characterize the evidence for renal lesions as a hazard of biphenyl exposure and to provide the rationale for selection of renal papillary mineralization as the critical effect.

3. Benchmark dose (BMD) modeling was conducted using the incidence of litters with fetal skeletal anomalies to estimate the point of departure (POD) for derivation of the RfD. Has the modeling been appropriately conducted and clearly described based on EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000a)? Is the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 10% extra risk of the incidence of litters with any fetal skeletal anomalies) supported and clearly described?

Comments: Four reviewers commented that the modeling was appropriately conducted, clearly described, or followed EPA guidance. One of these reviewers also commented that EPA's argument for not applying cross-species scaling to the oral dose for the developmental endpoint was problematic. Another reviewer emphasized the maternal toxicity at the high dose in the developmental study, and asked: (1) that the assessment be more clear regarding whether or not these data were included in the modeling, and (2) that justification be provided if these data were included. Two reviewers reiterated that the developmental study was not appropriate for RfD derivation, and the remaining reviewer noted his lack of familiarity with dose-response modeling.

Regarding BMR selection, two reviewers stated that the reason for using a BMR of 10% extra risk for incidence of litters with effects versus 5% among fetuses with effects was adequately explained, while two others commented that this selection should be explained further. The remaining four reviewers did not comment.

Response: As summarized under the first two charge questions, EPA agrees that the renal effects reported by Umeda et al. (2002) are more compelling for RfD derivation than the developmental effects reported by Khera et al. (1979). However, a candidate RfD for developmental toxicity was retained in the revised assessment in order to provide some perspective on the developmental hazard of biphenyl exposure. Following the reviewers' evaluation of the Khera et al. (1979) study, the dose-response analysis focused on missing or unossified sternebrae, the only anomaly that showed an increasing trend with dose in the absence of maternal toxicity. The high-dose group was omitted from dose-response modeling because of the demonstrated maternal toxicity. A modeling approach that approximates the result of nested models (due to the unavailability of detailed data showing the distribution of fetuses among litters) was implemented that enabled using a BMR of 5% extra risk among fetuses, precluding the need to

consider an equivalent degree of effect in terms of litter incidence. Briefly, BMD analyses used the proportions of affected fetuses within each dose group, and alternately used the total number of fetuses and the total number of litters as the group sizes to bracket the BMD and BMDL expected to result from a nested analysis of individual data, if they were available (see, e.g., Rao and Scott, 1992). Section 5.1.2 was revised to reflect this change.

EPA agrees that a body weight scaling to the ¾ power (i.e., $BW^{3/4}$) approach should be applied to extrapolate equivalent doses from dams to humans for the purpose of calculating a human equivalent dose, consistent with EPA guidance (U.S. EPA, 2011). Also consistent with this guidance, $BW^{3/4}$ scaling was used to extrapolate to human-equivalent doses for the renal endpoints. Detailed calculations can be found in Section 5.1.2.

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs appropriate based on the recommendations described in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

Comments: Five of the eight reviewers generally agreed with the selection of UFs applied to the POD for the derivation of the RfD; one of these reviewers further observed that the UFs were consistent with EPA guidance (U.S. EPA, 2002). Two reviewers did not offer comments because the topic was outside their area of expertise.

The remaining reviewer agreed with the UFs applied for interspecies and intraspecies adjustments, but recommended further discussion of the UFs for LOAEL to NOAEL extrapolation and for database deficiencies. Specifically, more discussion was recommended to support the justification for a LOAEL to NOAEL UF of 1 based on skeletal anomalies and the assumption that an effect at the BMDL represented a minimally biologically significant change. In addition, this reviewer suggested that the database UF of 1 could be raised to 3 or 10 because some animal studies were limited by small numbers of animals, incomplete histopathology, and insufficient study length and because the database lacked animal studies examining neurological effects (which were observed in workers) and developmental neurological effects.

Response: For the LOAEL to NOAEL UF assigned to the POD for developmental effects, EPA considered (in the draft assessment) an increase of 10% (extra risk) in incidence of litters with skeletal anomalies to be a change with minimal biological significance, because of its expected equivalence to a 5% extra risk in incidence of fetuses with skeletal anomalies. The revised analyses use a 5% extra risk BMR for incidence of missing or unossified sternebrae among fetuses, and a 10% extra risk BMR for the renal effects, both of which are judged to characterize minimally biologically significant changes.

The database UF of 1 for the oral RfD is supported, in part, by two chronic oral toxicity studies in rats and mice by Umeda et al. (2005) and Umeda et al. (2002) that were conducted according to OECD testing guidelines and conformed to OECD GLP principles. As noted by one reviewer, some animal studies were limited by small numbers of animals, incomplete histopathology, or insufficient study length. Nevertheless, these studies generally support the findings of the more robust studies by Umeda and colleagues, and as such, do not represent database deficiencies. Potential neurological effects of biphenyl were examined in two epidemiological studies of workers in two factories manufacturing biphenyl-impregnated paper. Information was not available to characterize biphenyl exposure quantitatively in either study, although workers from both factories were exposed to biphenyl at levels above the occupational limit of 1.3 mg/m^3 (threshold limit value [TLV] by ACGIH, 2001b), and in one of the two studies, an average air concentration almost 100 times the TLV was reported in one location in the plant. It is unclear how the findings from these workplace studies that predominantly involved inhalation exposure would relate to oral exposure. As noted by one reviewer, animal studies did not include examination of sensitive measures of neurotoxicity. The 2-year oral bioassays in rats and mice (Umeda et al., 2005; Umeda et al., 2002) did, however, include daily observations for clinical signs and histopathological examination of nervous system tissues. No nervous system effects were reported, suggesting that the nervous system is not a sensitive target of oral biphenyl toxicity. In summary, the findings from studies of occupational (predominantly inhalation) exposure to biphenyl introduce some uncertainties in the characterization of biphenyl hazard. These uncertainties are discussed in the justification for the database UF for the oral RfD in Section 5.1.3; however, EPA did not consider the uncertainties sufficient to warrant a database UF more than 1 in deriving the RfD.

(B) Inhalation Reference Concentration (RfC) for Biphenyl

1. The draft "Toxicological Review of Biphenyl" did not derive an RfC. Has the justification for not deriving an RfC been clearly described in the document? Are there available data to support the derivation of an RfC for biphenyl? If so, please identify these data.

Comments: All reviewers agreed that there are insufficient data to derive an inhalation RfC for biphenyl and that the justification for not deriving an RfC was clearly and adequately described. One reviewer specifically recommended against extrapolating from the oral value to derive an RfC because biphenyl pharmacokinetics may be relatively complicated and no data on route differences in pharmacokinetics are available. One reviewer disagreed with the text on page 89 stating "The lack of adequate data to derive an RfC represents a significant uncertainty for the evaluation of risks from exposure to inhaled biphenyl," and recommended that EPA compare

ambient air biphenyl concentrations with the TLV to provide perspective on likely risks from biphenyl inhalation.

Response: Consistent with the recommendations of the peer reviewers, an RfC for biphenyl was not derived. With regard to the recommendation to use the TLV as a point of comparison, it should be noted that this value applies to healthy adult workers and does not take into consideration effects of the chemical in children and other potentially susceptible lifestages and populations. Established in 1972, the TLV of 0.2 ppm (1 mg/m^3) was based on a subchronic mouse study conducted in 1947 (Deichmann et al., 1947) that showed respiratory effects at 1 ppm (6 mg/m^3). Thus, the TLV was established at a level only fivefold lower than the air concentration producing effects in the mouse. For the above reasons, the biphenyl TLV is not considered to be a health-protective value for general population exposures. In light of the comment, however, the text in Section 5.3 regarding potential risks of inhaled biphenyl was revised.

(C) Carcinogenicity of Biphenyl

1. Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the draft "Toxicological Review of Biphenyl" concludes that the database for biphenyl provides "suggestive evidence of carcinogenic potential" by all routes of exposure. Please comment on whether this characterization of the human cancer potential of biphenyl is scientifically supported and clearly described.

Comments: Three reviewers agreed with the cancer descriptor of "suggestive evidence of carcinogenic potential" for biphenyl. One of these reviewers characterized the liver tumor response in female BDF_1 mice (Umeda et al., 2005) as robust and as a sufficient basis in and of itself to support the suggestive descriptor. This reviewer also suggested that studies of durations not sufficiently long to be informative for carcinogenicity determination (including Dow Chemical Co, 1953; Monsanto, 1946) be excluded from this discussion and that deficiencies and limitations of other studies (including Shiraiwa et al., 1989; Ambrose et al., 1960; Pecchiai and Saffiotti, 1957) be further discussed.

One reviewer commented that the rationale for the cancer characterization should be more clearly described in Section 4.7, including identifying study limitations of Imai et al. (1983), strengthening the argument that humans are less susceptible to urinary bladder tumors, and making more explicit whether or not urinary bladder tumors were excluded in selecting the descriptor such that the positive tumor findings for biphenyl carcinogenicity apply to only one species, sex, strain, and site, thereby obviating the "likely to be carcinogenic" category.

One reviewer did not agree with the descriptor and recommended instead the term "some evidence" of carcinogenicity consistent with the terminology from NTP.

Three reviewers did not indicate whether or not they agreed with the selection of the suggestive descriptor. One of these reviewers observed that there was not enough synthesis of the data or attention paid to confounding (e.g., palatability, weight loss).

Response: EPA retained the cancer descriptor of "suggestive evidence of carcinogenic potential" for biphenyl and expanded the consideration of factors influencing the weight of evidence for carcinogenicity in Sections 4.7.1 and 4.7.2. EPA agreed that the absence of a tumor response in 1-year dog (Monsanto, 1946) and monkey (Dow Chemical Co, 1953) studies should not be considered in evaluating the cancer weight of evidence because the study durations were not sufficiently long and the group sizes (1–2 animals/sex/group) were too small to allow for detection of tumors. These two studies were excluded from the discussion. More thorough characterization of other studies that found no evidence of carcinogenic response (including study limitations) was added to Sections 4.7.1 and 4.7.2.

EPA's choice of weight-of-evidence descriptors is currently limited to one of the five weight-of-evidence descriptors identified in its *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). "Some evidence of carcinogenicity," used by NTP, is not among these five descriptors, and the cancer descriptor of "suggestive evidence of carcinogenic potential" was retained.

No treatment-related changes in food consumption or palatability that could have potentially confounded the results of the most informative studies of biphenyl carcinogenicity, i.e., Umeda et al. (2005); Umeda et al. (2002), were identified. For all studies, food consumption and body weight information, where available, were used in calculating doses in mg/kg body weight-day.

Comments: One reviewer considered EPA's treatment of bladder tumor findings as not contributing to the positive evidence at "environmentally relevant dose" to be well described, but also proposed that an alternative approach would be to address the issue of high-dose carcinogenicity via calculi formation leading to higher overall evidence for carcinogenicity in this dose region. Two reviewers did not agree with adding the language "at environmentally relevant exposure levels in humans" in the context of bladder tumors. One reviewer recommended more discussion of what constitutes "environmentally relevant exposure" in the context of bladder tumors.

Response: EPA agrees with the peer reviewers that the phrase "environmentally relevant exposures" is not particularly clear or helpful language. To be more clear and specific, EPA revised the text in Section 4.7.1 such that the descriptor was not tied to ranges of exposure.

Comment: One reviewer considered the discussion of evidence by route of exposure to have been well laid out, but suggested noting that Sun (1977a) provides evidence of distal impacts in the liver and kidney from inhalation exposure. Three reviewers questioned the application of the suggestive descriptor to all routes of exposure, noting that the data supporting toxicity/ carcinogenicity by routes of exposure other than oral was scanty or absent.

Response: Evidence of distal effects of inhaled biphenyl was reported in studies by Deichmann et al. (1947) and Sun (1977a); the discussion of carcinogenic potential by other routes of exposure in Section 4.7.1 was expanded to include these studies as indirect support for absorption of inhaled biphenyl. Evidence of dermal absorption of biphenyl is provided in an unpublished in vitro study (DuPont, 2005) submitted during the public comment period. Sections 3.1 and 4.7.1 were revised to include reference to this study. According to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), "[W]hen tumors occur at a site other than the point of initial contact, the descriptor generally applies to all exposure routes that have not been adequately tested at sufficient doses. An exception occurs when there is convincing information, e.g., toxicokinetic data that absorption does not occur by another route." Given the evidence, albeit limited, for absorption of biphenyl by inhalation and dermal routes, application of the descriptor to all routes of exposure was retained consistent with Agency guidance.

Comment: One reviewer commented that justification for the position that certain minor metabolites of biphenyl do not contribute to tumorigenesis was not adequate.

Response: EPA agrees that evidence for the genotoxicity of the metabolites of biphenyl was not adequately characterized. The discussions of the evidence for genotoxic vs. mutagenic activity in Section 4.5.2 and Appendix C were revised, and the evidence for a mutagenic mode of action based on data for biphenyl and its metabolites was clarified in Section 4.7.3.1.3.

2. EPA has concluded that biphenyl-induced urinary bladder tumors in male rats 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. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available that may support an alternative mode of action for biphenyl-induced urinary bladder tumors.

Comments: Six of the eight reviewers agreed that the proposed mode of action for biphenyl-induced urinary bladder tumors was supported and clearly described. One of these reviewers observed that a small contribution to urinary bladder carcinogenesis from genotoxic biphenyl metabolites cannot be ruled out, but that this possibility did not preclude a conclusion that calculi formation was required for the observed bladder tumors.

Two reviewers did not consider the mode of action to be sufficiently supported. One of these reviewers commented that data did not prove that bladder stones were required for carcinogenesis and biphenyl may cause both stones and cancer, not necessarily in any specific order. However, this reviewer stated that he was not aware of another proven mode of bladder carcinogenesis for biphenyl. The second reviewer did not consider the explanations for gender- and species-specific association between bladder calculi formation and development of bladder tumors to be clear, questioned whether there had been exploration of alternative mechanisms of action, and suggested consideration be given to an alternative mode of action based on data for 2-aminobiphenyl for which there is evidence of up-regulation of the expression of COX-2 via NADPH oxidase-derived ROS-dependent pathways in a bladder cancer cell line.

Response: Regarding the contribution of biphenyl metabolites to a mutagenic mode of action, see the response under Charge Question C.1.

EPA retained the hypothesized mode of action for biphenyl-induced urinary bladder tumors because the available data demonstrated a strong, consistent, and specific association between calculi formation and urinary bladder tumor occurrence. As discussed in Section 4.7.3.1 and consistent with the cancer mode-of-action framework provided in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the key events in the hypothesized mode of action (i.e., calculi formation followed by irritation of transitional epithelial cells of the urinary bladder, sustained cell proliferation, and promotion of initiated cells in the urinary bladder with progression to papillomas and carcinomas) show dose-response concordance, a temporal relationship, and biological plausibility.

The available information on gender- and species-specific differences in calculi formation and development of bladder tumors presented in Section 4.7.3.1.2 was revised to clarify the gender differences in calculi formation and tumor response. As discussed in this section, the differences in calculi formation (i.e., lack of calculi in mice and the differences in chemical composition and physical properties of calculi between male and female rats) is consistent with the lack of urinary bladder tumor response in mice and female rats. An alternative mode of action for biphenyl based on a mechanistic study of 2-aminobiphenyl was not included in the Toxicological Review because 2-aminobiphenyl is not a metabolite or precursor of biphenyl and the relevance of the findings of this study to biphenyl is not clear.

Comments: One reviewer considered the term "transitional cell carcinoma" to be outdated and recommended using instead the current terminology—"urothelial carcinoma" (WHO, 2004; Epstein et al., 1998).

Response: The term "urothelial" specifically refers to a carcinoma of the urothelium, meaning a transitional cell carcinoma of the urinary system. Currently, "transitional cell carcinoma" and "urothelial carcinoma" are used interchangeably. To be consistent with the term used by Umeda et al. (2002), the term "transitional cell carcinoma" was retained.

3. EPA has concluded that there is insufficient information to identify the mode(s) of carcinogenic action for biphenyl-induced liver tumors in mice. Please comment on whether this determination is appropriate and clearly described. If it is judged that a mode of action can be established for biphenyl-induced mouse liver tumors, please identify the mode of action and its scientific support (i.e., studies that support the key events, and specific data available to inform the shape of the exposure-response curve at low doses).

Comments: All reviewers agreed that there is insufficient information to identify the mode(s) of carcinogenic action for biphenyl-induced liver tumors in mice, and that this determination was appropriate and clearly described.

Response: No response is needed.

Oral Slope Factor (OSF)

4. A two-year cancer bioassay of biphenyl in BDF_1 mice (Umeda et al., 2005) was selected as the basis for the derivation of the OSF. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the OSF, please identify this study and provide scientific support for this choice.

Comments: Seven reviewers agreed with the selection of the Umeda et al. (2005) study as the basis for the derivation of the oral slope factor, generally noting that the rationale was clearly described and scientifically supported. One of these reviewers suggested including more detailed explanation and evaluation of the strengths and weakness of other studies in mice and other species to assess the entire set of relevant data. The eighth reviewer recommended selecting the study with the lowest NOAEL for the derivation of the oral slope factor.

Response: A discussion of strengths and weakness of the available chronic bioassays for biphenyl was provided in Section 4.7.2; text was added to Section 5.4.1 directing the reader to that discussion.

An oral slope factor describes the cancer risk per unit dose of the chemical at low doses. Unless a mode of action consistent with nonlinear extrapolation is established, the assumption is made that the relationship between risk of cancer and exposure is linear, i.e., there is some risk of cancer at all exposures to the chemical. Under this assumption, a NOAEL for cancer cannot be identified. Therefore, selection of the study with the lowest NOAEL was not an appropriate consideration for deriving the OSF for biphenyl.

5. The incidence of liver tumors (i.e., adenomas or carcinomas) in female mice was selected to serve as the basis for the derivation of the OSF. Please comment on whether this selection is scientifically supported and clearly described. If a different cancer endpoint is recommended for deriving the OSF, please identify this endpoint and provide scientific support for this choice.

Comments: Two reviewers agreed that liver tumor incidence in female mice was the most appropriate data set for the derivation of the oral slope factor. One reviewer considered the rationale for the selection of female liver tumors to be clearly described.

One reviewer commented that the discrepancy in liver tumor responses between male and female mice should be addressed in the discussion of data choices for dose-response analysis and in the discussion of implications for the usefulness of the oral slope factor. One reviewer commented that consideration of another study was warranted given the finding of liver tumors in female mice only with low incidence in the concurrent control group, but did not identify a more appropriate study.

One reviewer suggested that consideration be given to using urologic toxicity data given that liver tumors form more easily in mice, liver tumors occurred almost exclusively in female mice, urinary toxicity has been consistently observed in all studies at high levels, and bladder tumors were the common cause of animal death. This reviewer also acknowledged that liver toxicity was the predominant toxic effect in human studies.

Response: EPA agrees that the differing liver tumor responses for male and female BDF_1 mice merit further clarification and discussion in the assessment. The statistically significant decreasing trend in male mice was clarified in Sections 4.2.1.2.2 (Chronic mouse studies), 4.7 (Evaluation of Carcinogenicity), and 5.4.1 (Choice of Study/Data). Regarding the extent of difference between the two responses, the female mice showed a clearly positive response, with liver tumor incidence in the two highest dose groups exceeding the range of historical control data for that laboratory. The decreased responses in male mice suggested an anticarcinogenic

response; however, all responses in male mice were within the range of historical control data. Further, the concurrent dose-related decrease in male BDF_1 mouse body weight gain was similar to that in $B6C3F_1$ mice whose decreased trends in liver tumors were judged not to demonstrate anti carcinogenicity (Leakey et al., 2003; Haseman and Johnson, 1996). In the absence of an understanding of the mode of action of biphenyl hepatocarcinogenicity, the reason for the gender-related difference in response to induction of liver tumors by biphenyl is unknown. While an increase in tumor incidence in both males and females would increase the weight of evidence for carcinogenicity, concordance in tumor response across sexes is not necessarily expected, and the lack of a positive response in male BDF_1 mice does not invalidate the positive response observed in female mice. The health-protective assumption is made that the tumor response from the most sensitive gender is relevant to humans, and therefore liver tumor incidence in the female mouse from the Umeda et al. (2005) study served as the basis for the oral slope factor for biphenyl. The rationale for selecting female mouse liver tumor data for dose-response analysis was further clarified in Section 5.4.1. Uncertainty in the biphenyl oral slope factor associated with the selection of female mouse liver tumors as the basis for the slope factor was addressed in Section 5.4.5.1 and Table 5-11.

The occurrence of urinary bladder tumors in male rats chronically exposed to biphenyl in the diet is considered a high-dose phenomenon associated with calculi formation. No increased risk of bladder tumors is expected as long as exposure to biphenyl is below the dose needed to form calculi. Because the occurrence of urinary bladder tumors is considered to be nonlinear at low doses, derivation of an oral slope factor based on urologic toxicity data (in this case bladder tumor incidence data) is not supported.

Comments: One reviewer did not consider the rationale for combining adenoma and carcinoma data for the calculation of the oral slope factor to be well described, and suggested that adenoma data alone would be more appropriate since the carcinoma incidence at the high dose was not statistically different from control.

Response: Data are not available to indicate whether malignant tumors developed specifically from the progression of benign tumors in biphenyl-exposed female mice; however, etiologically similar tumor types (i.e., benign and malignant tumors of the same cell type) were combined for dose-response analyses because of the possibility that the benign tumors could progress to the malignant form (McConnell et al., 1986). This is consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), which state that "[t]he incidence of benign and malignant lesions of the same cell type, usually within a single tissue or organ, are considered separately but may be combined when scientifically defensible." The rationale for combining liver adenoma and carcinoma incidence for OSF derivation was added to Section 5.4.2.

6. Benchmark dose (BMD) modeling was conducted using the incidence of liver tumors in female mice in conjunction with dosimetric adjustments for calculating the human equivalent dose (HED) to estimate the point of departure (POD). A linear low-dose extrapolation from this POD was performed to derive the OSF. Has the modeling been appropriately conducted and clearly described based on EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000a)? Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 10% extra risk of the incidence of liver tumors in female mice) been supported and clearly described?

Comments: Six reviewers generally considered that the BMD modeling was appropriately conducted and clearly described. One reviewer stated that the BMD modeling approach was clearly described, but did not provide a critical assessment because modeling was outside the reviewer's area of expertise. Two of the reviewers specifically commented that the rationale for using 10% extra risk of the liver tumor incidence in female mice was well supported. One reviewer recommended changing the text on page 94 from "the multistage model" to "the multistage-cancer model." One reviewer offered no comment.

Response: It is technically correct that the "multistage model-cancer" was used for analysis of cancer data; however, the model is mathematically identical to the multistage model. Clarification was added as a footnote in Section 5.4.3.1.

7. EPA has concluded that a nonlinear approach is appropriate for extrapolating cancer risk from male rats to humans because the mode of action analysis suggests that rat bladder tumors occur only after a series of events that begin with calculi formation. At exposure levels below the RfD (i.e., below exposure levels needed to form calculi), no increased risk of cancer is expected. Please comment on whether this approach is scientifically supported and clearly described. Please identify and provide the rationale for any other extrapolation approaches that should be selected.

Comments: Six of the eight reviewers agreed with use of a nonlinear approach for extrapolating cancer risk from male rat bladder tumors to humans. One of these reviewers recommended a comparison between the RfD and the NOAEL for calculi formation since the RfD was derived from a developmental endpoint rather than calculi formation. One reviewer stated that modeling the bladder tumor endpoint is not needed since it was determined that these tumors would not occur at environmentally relevant doses. One reviewer observed that the data suggest, but do not prove, a multistep carcinogenic process for bladder tumors and considered bladder stones to be contributing, but not sufficient, to cause bladder cancer.

Response: EPA agrees with the reviewers who considered a nonlinear extrapolation approach for male rat bladder tumors to be supported; this approach was retained. A comparison of the candidate RfD that would be derived from the NOAEL for bladder calculi in the male rat (i.e., a key event in the mode of action for urinary bladder tumors) and the RfD based on renal toxicity of biphenyl in rats was added to the discussion of the nonlinear extrapolation approach for bladder tumors in Section 5.4.3.2. (As noted in response to comments under Charge Questions A.1 and A.2, the critical effect for the RfD was changed from a developmental endpoint to renal toxicity.)

To address concerns raised by two peer reviewers who questioned whether the key events in the mode of action for biphenyl-induced bladder tumors had been established, EPA added, as a part of the uncertainty analysis, a linear low-dose extrapolation approach to data for urinary bladder tumors in male rats. The resulting oral slope factor based on bladder tumors of 2×10^{-3} $(mg/kg\text{-}day)^{-1}$ is fourfold lower than the oral slope factor based on liver tumors of 8×10^{-3} $(mg/kg\text{-}day)^{-1}$. This analysis, which is presented in Section 5.4.5, Uncertainties in Cancer Risk Values, demonstrates that the oral slope factor derived from liver tumor data is protective of the OSF that would be derived from urinary bladder tumor data under the assumption that a linear extrapolation approach for bladder tumors was supported. The comment related to the role of calculi formation in bladder tumor carcinogenesis is addressed in a response under Charge Question C.2.

Inhalation Unit Risk (IUR)

8. The draft "Toxicological Review of Biphenyl" did not derive an IUR due to the lack of available studies. Are there available data to support the derivation of an IUR for biphenyl? If so, please identify these data.

Comments: None of the reviewers identified studies to support derivation of an inhalation unit risk. One reviewer observed that deriving an inhalation unit risk from the oral slope factor in the absence of inhalation pharmacokinetics would be uncertain.

Response: EPA agrees that use of route-to-route extrapolation to derive an inhalation unit risk is not supported.

II. Public Comments

EPA received two sets of public comments. One of these commenters observed that the draft *Toxicological Review of Biphenyl* as well written, concise, and made reasonable assertions based on the literature. Specific comments and responses are summarized below.

Comments: One public commenter stated that the toxicokinetics section (Section 3) was well written, but offered two recommendations for providing expanded detail:

> (1) Regarding the discussion of 2-hydroxybiphenyl (or *ortho*-phenylphenol) in Section 3.3.2.1, emphasis should be given to the fact that urinary bladder tumor formation following 2-hydroxybiphenyl exposure is dose-dependent and is observed only at high doses. The commenter provided the following citations: Reitz and Watanabe (1983); Reitz et al. (1983), and Smith et al. (1998).
> (2) A description of the potential redox cycling between 4,4'-dihydroxybiphenyl and 4,4'-dihydroxybiphenylquinone should be included for clarity and completeness.

Response: The sentence related to urinary bladder tumors associated with 2-hydroxybiphenyl was changed to provide a more accurate description of bladder tumor induction by this chemical, including the fact that the dose-response relationship is nonlinear (i.e., incidence of bladder tumors of 96% at 1.25% in diet, but no tumors at the lower concentration of 0.625%) (Kwok et al., 1999; Hiraga and Fujii, 1984). One of the studies by Reitz and colleagues and the Smith et al. (1998) study were already cited in the Toxicological Review; the second Reitz and Watanabe (1983) study did not contribute substantive new information to the Toxicological Review and was therefore not added. A focused literature search did not locate any studies on metabolism of 4,4'-dihydroxybiphenyl to the semiquinone and the potential redox cycling between 4,4'-dihydroxybiphenyl and 4,4'-dihydroxybiphenylquinone. Because this metabolic pathway is speculative, it was not included in the Toxicological Review.

Comments: One public commenter recommended that limitations of the Sun (1977a) inhalation study be reiterated in the summary of the noncancer endpoints, and that more clear explanations be added in Section 4.2, Subchronic and Chronic Studies and Cancer Bioassays in Animals—Oral and Inhalation, to clarify the reasons that some studies were considered more reliable than others. Another public commenter pointed out that the protocols used to evaluate the studies relied upon in the assessment were not defined.

Response: A summary of the limitations of the Sun (1977a) study was included in Section 4.6.2 and reiterated in Section 5.2.1. The evaluation of study quality was consistent with EPA guidance, including *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002). Reference to relevant agency guidance was added to Section 5.2.1. A new appendix, Literature Search Strategy and Study Selection, provides additional information on study selection strategy and identification of EPA guidance documents used to guide study evaluation.

Comments: Both public commenters stated that the critical effect selected for derivation of the RfD, fetal skeletal anomalies (missing, delayed, or unossified sternebrae), should be considered as a non-adverse variation, noting that delayed sternebrae ossification would be expected to fully ossify within a few days postnatally, and would have no impact on the viability or function of the offspring. These commenters cited Carney and Kimmel (2007) and Marr et al. (1992) as support. One of the public commenters also considered the delayed ossification to be secondary to maternal toxicity (reduced body weight). Therefore, the public commenters argued that delayed sternebrae ossification should not be the critical effect used to calculate the oral RfD.

Response: As discussed in responses under Charge Questions A.1 and A.2, EPA agrees that the uncertainties in the interpretation of fetal skeletal anomalies reported in Khera et al. (1979) as an adverse effect were not adequately weighed in selecting this endpoint as the critical effect for the RfD, and discussion of the issues associated with interpretation of these anomalies was expanded in Sections 4.3.1 and 4.6.1. Among the anomalies listed, missing or unossified sternebrae was the only endpoint elevated with increasing dose at doses lower than 1,000 mg/kg-day (i.e., the dose associated with maternal toxicity), and this endpoint was retained as a candidate critical effect.

Consistent with peer reviewer recommendations, the robust toxicity studies in rats and mice by Umeda et al. (2005); Umeda et al. (2002) were also considered as candidate principal studies, with the rationale clarified in Section 5.1.1. Consistent with peer reviewer recommendations, renal lesions, and in particular renal papillary mineralization in male rats, was selected as the critical effect. The rationale for selection of this critical effect is provided in Section 5.1.2.

Comments: One public commenter offered the determination that the in vitro genotoxicity evaluation of biphenyl was negative to slightly equivocal and the in vivo data were negative, implying that biphenyl is not genotoxic.

Response: EPA disagrees with the conclusion that biphenyl is not genotoxic, although overall, there is not enough evidence to conclude that biphenyl is mutagenic or can react directly with DNA. The discussions in Section 4.5.2 and Appendix C were revised to more precisely characterize the evidence for the genotoxicity of biphenyl and its metabolites.

Comments: One public commenter agreed with the overall conclusion that bladder tumors (Section 4.7) are secondary to calculi formation and are not caused by a genotoxic mode of action. This commenter recommended that a discussion of the reversibility of calculi formation as reported by Booth et al. (1961) be added to Section 4.7.

Response: Booth et al. (1961) reported that urine volume, urine turbidity, and histopathological lesions, including focal tubular dilation and cellular fibrous tissue formation, were increased in male albino rats exposed to biphenyl in the diet for 120 days compared to controls. After exposure was stopped and the rats were fed a control diet for 30 days, the severity of these effects decreased. Effects mostly disappeared after being on the control diet for 60 days. The formation of calculi was not reported in the study. Although reversibility of kidney lesions was observed, this study did not directly demonstrate that calculi formation was reversible. Therefore, this study was not included in the discussion of mode of action of bladder tumors (Section 4.7).

Comment: One public commenter pointed to chronic studies that provided no evidence that biphenyl is carcinogenic 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), and Rhesus monkeys (Dow Chemical Co, 1953), and argued that the total weight of evidence of biphenyl carcinogenicity should be "inadequate information to assess carcinogenic potential" based on EPA guidance that states that where there is "conflicting evidence—that is—some studies provide evidence of carcinogenicity but other studies of equal quality in the same sex and strain are negative." Another public commenter observed that in light of the susceptibility for liver tumors in female mice, the negative carcinogenicity findings in male mice in the Umeda et al. (2005) bioassay and in mice in other studies (Imai et al., 1983; NCI, 1968), and absence of a carcinogenic response in chronic assays in dogs and monkeys, the cancer descriptor of "suggestive evidence of carcinogenic potential" was not supported. This commenter also stated that EPA did not define the protocols used to evaluate the studies relied on in the assessment, in particular with respect to the determination that the negative chronic bioassays in mice, rats, dogs, and monkeys published between 1946 and 1989 were less informative.

Response: According to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a descriptor of "inadequate information to assess carcinogenic potential" may be appropriate when there is "conflicting evidence, that is, some studies provide evidence of carcinogenicity but other studies of equal quality in the same sex and strain are negative." Earlier studies of biphenyl that provided no evidence of carcinogenicity used more limited study designs, including less-than-lifetime exposure durations, relatively small numbers of animals, or low doses and were therefore less informative than the more recent studies by Umeda et al. (2005) and Umeda et al. (2002). The limitations of these earlier studies are noted in Section 4.7.1 and summarized in more detail in Section 4.7.2. In light of the overall weight of evidence, EPA retained the cancer descriptor of "suggestive evidence of carcinogenic potential" for biphenyl.

EPA guidelines for evaluation of study quality are discussed in responses under General Charge Question 1 and Charge Question C.1.

Comment: One public commenter noted that in light of the limited inhalation and dermal exposure data in animals and humans, the cancer descriptor was not justified for all routes of exposure. A second public commenter submitted an unpublished reported performed by E.I. du Pont de Nemours and Company's Haskell Laboratory for Health and Environmental Sciences entitled "Biphenyl: In Vitro Dermal Absorption Rate Testing" (DuPont, 2005) and recommended that this study be considered in the determination of potential carcinogenicity by non-oral routes of exposure in Section 4.7.1.

Response: The study by DuPont (2005) measured human skin penetration rates of biphenyl using an in vitro skin culture system. This study was added to Sections 3.1 and 4.7.1 as evidence that biphenyl can be absorbed by dermal exposure. Inhalation toxicity studies in rats and mice reported systemic (liver and kidney) effects, and provided qualitative evidence for absorption of inhaled biphenyl (Sun, 1977a; Deichmann et al., 1947; Monsanto, 1946). As discussed in a response under Charge Question C.1, the discussion of biphenyl's carcinogenic potential by other routes of exposure (Section 4.7.1) was revised to better support the cancer descriptor of "suggestive evidence of carcinogenic potential" by all routes of exposure.

Comments: One public commenter recommended using the 2-year bioassay by Umeda et al. (2002) as the principal study for derivation of the RfD, noting that it was conducted according to OECD Guideline 453 and yielded the lowest NOAEL of 38 mg/kg-day (calculated from the dietary concentration of 500 ppm) of the five available dietary studies.

Response: Consistent with comments from external peer reviewers and the public, the principal study was changed from Khera et al. (1979) to Umeda et al. (2002). See response under Charge Questions A.1 and A.2 for additional discussion of the basis for this revision.

Comment: One public commenter supported the decision to use a nonlinear dose-response analysis for biphenyl-induced urinary bladder tumors.

Response: No response necessary.

Comment: One reviewer submitted three unpublished studies: (1) Cytogenetic Effects of Diphenyl-99 on Rat Bone Marrow Cells (conducted by Toxicology Research Laboratory, undated), (2) Biphenyl: In Vitro Dermal Absorption Rate Testing (conducted by Haskell Laboratory for Health and Environmental Sciences, 2005), and (3) Evaluation of Biphenyl FP in

the Mouse Bone Marrow Micronucleus Test (conducted by Toxicology & Environmental Research and Consulting, Dow Chemical Company, 2007).

Response: These studies were added to the Toxicological Review.

APPENDIX B. LITERATURE SEARCH STRATEGY AND STUDY SELECTION

The literature search strategy used to identify primary, peer-reviewed literature pertaining to biphenyl was conducted using the databases and keywords listed in Table B-1. References from health assessments developed by other national and international health agencies were also examined. Other peer reviewed information, including review articles, literature necessary for interpretation of biphenyl-induced health effects, and independent analyses of health effects data were retrieved and included in the assessment where appropriate. EPA requested public submissions of additional information in December 2007; no submission in response to the data call-in were received. A comprehensive literature search was last conducted in September 2012.

Figure B-1 depicts the literature search, study selection strategy, and the number of references obtained at each stage of literature screening for all searches. A total of 3,682 references were obtained from the literature searches. A more detailed manual review of titles, abstracts, and/or papers was then conducted. Selection of studies for inclusion in the Toxicological Review was based on consideration of the extent to which the study was informative and relevant to the assessment and general study quality considerations. In general, relevance and study quality was evaluated as outlined in EPA guidance, including *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhaled Dosimetry* (U.S. EPA, 1994b). The reasons for excluding references identified by the search are provided in Figure B-1. A preliminary manual screening of titles and abstracts determined that 3,398 studies were not relevant to the toxicity of biphenyl. Based on evaluation of the abstracts and full papers for the 284 considered references, 126 additional references were further eliminated.

The available studies examining the health effects of biphenyl exposure in humans and laboratory animals are discussed and evaluated in the hazard identification sections of the assessment (Section 4), with specific limitations of individual studies and of the collection of studies noted.

The references considered and cited in this document, including bibliographic information and abstracts, can be found on the Health and Environmental Research Online (HERO) website[10] (http://hero.epa.gov/biphenyl).

[10]HERO is a database of scientific studies and other references used to develop EPA's risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 300,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

Table B-1. Details of the literature search strategy employed

Database	Keywords
2007 Search	
Pubmed Toxline Biosis Embase	Chemical CASRN: 92-52-4 Synonyms: Biphenyl, Diphenyl, 1,1'-Biphenyl, 1,1'-Diphenyl, Bibenzene, Biphenyl, Lemonene, Phenylbenzene, Xenene Chemical CASRN: 8004-13-5 Synonyms: therminol vp-1, dowtherm A, dinil, dinyl, or diphyl PubMed: toxic* Toxline: standard terms such as toxic, genotoxic, developmental, etc. Biosis and Embase: toxic, toxico?, toxicit?, chronic, subchronic, acute, oral, inhale?, inhalation, dermal, intravenous, cancer?, carcinog?, carcinoma?, oncogene?, tumor?, neoplasm?, mutag?, mutat?, genotox?, fetotox?, embryotox?, teratology?, teratogen?, reproductive, developmental, neurotox?, immunotox?, pharmacokinetic?, pharmacodynamic?, PBPK, metabolism, epidemiol?, human study, and human studies
2008 and 2012 Searches	
Pubmed Toxcenter Toxline Current Contents	Chemical CASRN: 92-52-4 Synonyms: Biphenyl, Diphenyl, 1,1'-Biphenyl, 1,1'-Diphenyl, Bibenzene, Biphenyl, Lemonene, Phenylbenzene, Xenene Standard toxicology (all databases) Toxicity (including duration, effects to children and occupational exposure); development; reproduction; teratogenicity; exposure routes; pharmacokinetics; toxicokinetics; metabolism; body fluids; endocrinology; carcinogenicity; genotoxicity; antagonists; inhibitors Chemical specific (all databases) Further limited searches as needed to remove terms related to large classes of chemicals (PCB, PBDD, etc.) especially when searching for synonyms
TSCATS	Searched by chemical names (including synonyms) and CASRNs
ChemID	
Chemfinder	
CCRIS	
HSDB	
GENETOX	
RTECS	
HERO	Downloaded items already tagged to biphenyl

CCRIS = Chemical Carcinogenesis Research Information System; HSDB = Hazardous Substances Data Bank; RTECS = Registry of Toxic Effects of Chemical Substances; TSCATS = Toxic Substances Control Act Test Submissions

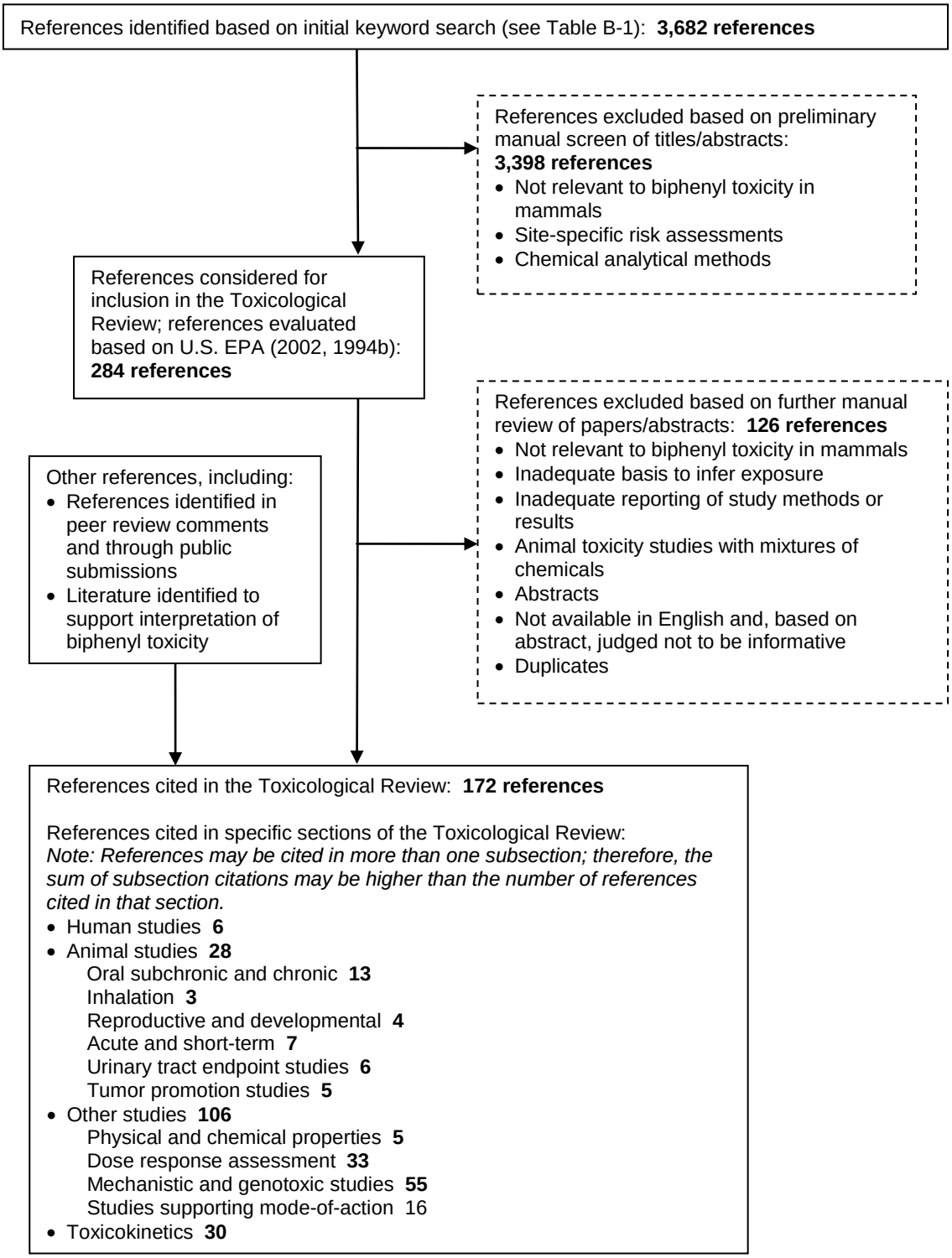


Figure B-1. Study selection strategy.

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

C.1. EFFECTS ON THE URINARY BLADDER OF RATS

Urinary bladder 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 et al. (2000a), Ohnishi et al. (2001) and Ohnishi et al. (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 16,000 ppm 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 C-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.

Table C-1. Content of biphenyl sulphate conjugates in urine and urinary crystals from male 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).

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). In addition to differences in chemical composition, Ohnishi et al. (2000a) observed that the physical appearance of calculi, including shape, size, and color, differed between male and female rats. Table C-2 compares the physical characteristics and major chemical constituents of calculi from male and female rats.

Table C-2. Comparison of the physicochemical characteristics of urinary calculi in male and female F344 rats

Property	Male	Female
Shape	Spheroid, triangular pyramidal, cubical	Spheroid
Size	0.3-1.0 cm	Homogeneous
Color	White, yellow, brown, gray, black	White, yellow
Main constituent	Potassium 4-hydroxybiphenyl-O-sulphate	4-Hydroxybiphenyl and potassium sulfate

Source: Umeda et al. (2005); Ohnishi et al. (2000b).

In the Ohnishi et al. (2000b) study, the pH of the urine of treated male rats was in the range of 7.5–8.5 during the last week of exposure, whereas in female rats, it was in the range of 6.5–8.0; there was no difference in urine pH between male and female controls (the range for both was 6.5–8.0). To investigate if pH of the urine was the only factor associated with calculi formation, Ohnishi et al. (2001) added potassium bicarbonate (5%), potassium chloride (5%), or sodium bicarbonate (8%) to the diet for 13 weeks. and 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.

C.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., 2004a). 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. Identification of peroxisomes was based on 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. Electon microscopy was limited to tissues from two female mice in the control and 16,000-ppm groups. 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 mice.

To examine the effects of biphenyl on hepatic peroxisomal enzyme and drug-metabolizing enzyme activities, Sunouchi et al. (1999) administered biphenyl to BDF_1 mice at oral doses of 1.3, 2.6, and 5.2 mmol/kg for 3 days. In female mice, biphenyl administration was associated with increases in potassium cyanide-insensitive PCO oxidation in liver homogenates (up to 1.9-fold), lauric acid 12-hydroxylation in liver microsomes (up to 3.8-fold), and cytochrome P450 protein level (as determined by immunochemical analysis). PCO oxidation and LA 12-hydroxylation were not affected in biphenyl-exposed male mice. Administration of biphenyl (5.2 mmol/kg) increased PROD (1.8-fold in females; 2.3-fold in males) and P450 protein level (as determined by immunochemical analysis). Relative liver weights were not affected. This study was reported as an abstract only; additional study details were not provided.

C.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-, and 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.

C.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.

C.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.

C.6. GENOTOXICITY

Biphenyl. The results of genotoxicity studies of biphenyl are summarized in Table C-3 at the end of this section. In bacterial systems, 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 (S9 rat liver microsomal fraction). Biphenyl did not appear to induce DNA repair in the SOS chromotest in *E. coli* (Brams et al., 1987), in the host-mediated assay in *E. coli* (Hellmér and Bolcsfoldi, 1992), or in the recombinational repair assay in *Bacillus subtilis* (Garrett et al., 1986; Kojima and Hiraga, 1978), with or without the presence of S9. In yeasts, biphenyl did induce mitotic recombination and gene conversion both with and without S9 in *Saccharomyces cerevisiae* strain D7 (Pagano et al., 1988).

Assays for gene and chromosomal mutations of biphenyl-exposed cultured mammalian cells demonstrate some ability of biphenyl to induce mutagenicity in these systems. Glatt et al. (Glatt et al., 1992) observed hprt mutations in Chinese hamster V79 cells, but only when cultured with NADPH-fortified S9 mix. Biphenyl also induced forward mutations in mouse L5178Y/$TK^{+/-}$ lymphoma cells (Wangenheim and Bolcsfoldi, 1988). The mutation frequency was increased two- to fourfold in the 10–20% total growth range only, leading the authors to consider biphenyl to be weakly mutagenic, even though this result was still within study guidelines for a positive result ($p \leq 0.001$).

A study of human primary peripheral blood cells reported significant increases in chromosomal aberrations (two- to fourfold higher than solvent controls), micronuclei

(approximately 2.5-fold higher than solvent controls), and sister chromatid exchanges (SCEs) (less than twofold higher than solvent controls) without S9 that increased with dose (Rencüzoğullari et al., 2008). These results, however, were accompanied by dose-dependent cytotoxicity (measured as a reduction in cell replication indices) that was significant at the two highest doses. Abe and Sasaki (1977) showed a nearly twofold increase in chromosomal aberrations at 30 μg/mL and a statistically significant increase in SCEs at 15 μg/mL (pairwise t-test) in Chinese hamster lung cells without activation, but these responses did not meet the authors' criteria for a positive result due to a lack of dose response. Ishidate et al. (1984) did not find an increase in chromosomal aberrations up to 125 μg/mL in the same cell line, in agreement with other studies (Abe and Sasaki, 1977) and their own past results (Ishidate and Odashima, 1977). However, the same group subsequently performed the same analysis in the presence of S9 and obtained positive results that increased with dose (Sofuni et al., 1985).

In the only study to quantify DNA strand breaks, Garberg et al. (1988) found a significant increase in DNA breakage as detected by the alkaline elution assay in mouse lymphoma cells. None of the studies for the detection of unscheduled DNA synthesis (Hsia et al., 1983b, a; Probst et al., 1981; Brouns et al., 1979; Williams, 1978) in biphenyl-treated rat liver cells reported positive results, however, indicating that no DNA excision repair was taking place. A report of the cell transformation assay in human and hamster cells was also negative (Purchase et al., 1978).

Evaluations of the potential genotoxicity of biphenyl in vivo have been performed in rats and mice. Two investigations of chromosomal mutations found no evidence of an increase in chromosomal aberrations in rats following inhalation exposure to biphenyl dust (Dow Chemical Co, 1976) or of micronuclei in mouse bone marrow after a single gavage dose (Dow Chemical Co, 2007). One group, however, did find evidence of DNA strand breaks in mice using the 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).

Table C-3. Genotoxicity test results for biphenyl

Endpoint	Strain or test system	Dose/ concentration[a]	Results		Reference
			+S9	–S9	
Prokaryotic organisms					
Reverse mutation	*S. typhimurium* TA98, TA100	2 µg/plate	–	–	Houk et al. (1989)
	S. typhimurium TA98, TA100	25 µg/plate	–	NT	Bos et al. (1988)
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, TA1978	77 µg/plate	–	–	Westinghouse Electric Corporation (1977)
	S. typhimurium TA98, TA100, TA1535, TA1537	100 µg/plate	–	–	Haworth et al. (1983)
	S. typhimurium TA97, TA98, TA100	100 µg/plate	–	–	Brams et al. (1987)
	S. typhimurium TA98, TA100, YG1041	250 µg/plate	–	–	Chung and Adris (2003, 2002)
	S. typhimurium TA98, TA100, TA1532, TA1535, TA1537, TA1538, TA2636	500 µg/plate	–	–	Pagano et al. (1988); Pagano et al. (1983)
	S. typhimurium TA98, TA100	800 µg/plate	–	–	Glatt et al. (1992)
	S. typhimurium TA98, TA1535	1,000 µg/plate	–[b]	NT	Narbonne et al. (1987)
	S. typhimurium TA98, TA100	1,000 µg/plate	–	–	Kojima and Hiraga (1978)
	S. typhimurium TA98, TA100, TA1535, TA1537	2,500 µg/plate	–	NT	Purchase et al. (1978)
	S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537, TA2637	5,000 µg/plate	–	–	Ishidate et al. (1984)
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, C3076, D3052, G46	1,000 µg/mL	–	–	Cline and Mcmahon (1977)
	S. typhimurium C3076, D3052, G46, TA98, TA1000, TA1535, TA1537, TA1538	10^4-fold range	–	–	Probst et al. (1981)
	E. coli WP2, WP2*uvr*A	1,000 µg/mL	–	–	Cline and Mcmahon (1977)
	E. coli WP2, WP2*uvr*A	10^4-fold range	–	–	Probst et al. (1981)
	E. coli WP2	1,000 µg/mL	–	–	Kojima and Hiraga (1978)
DNA repair	*E. coli* PQ37 SOS chromotest	154 µg/mL	–	–	Brams et al. (1987)
Differential DNA repair	*E. coli* K-12 uvrB/recA$^+$, K-12 uvrB/recA$^-$ Host-mediated assay	25,000 µg/mL	–	–	Hellmér and Bolcsfoldi (1992)
DNA recombination/ repair	*Bacillus subtilis* rec assay H17 (rec$^+$), M45 (rec$^-$)	10,000 µg	–	–	Kojima and Hiraga (1978)
Non-mammalian eukaryotic organisms					
Mitotic recombination	*S. cerevisiae* D7	1.5 µg/mL[c]	+ (DR)	+ (DR)	Pagano et al. (1988)
Gene conversion	*S. cerevisiae* D7	1.5 µg/mL[c]	+ (DR)	+ (DR)	

Table C-3. Genotoxicity test results for biphenyl

Endpoint	Strain or test system	Dose/ concentration[a]	Results		Reference
			+S9	–S9	
Mammalian cells in vitro					
Mutation	Chinese hamster V79 cells hprt locus	25 µg/mL[d]	+ (DR)	–	Glatt et al. (1992)
	Mouse lymphoma L5178Y cells tk locus	3.1 µg/mL	±[e] (T)	– (T)	Wangenheim and Bolcsfoldi (1988, 1986)
Micronuclei	Human primary peripheral blood lymphocytes	30 µg/mL[f]	NT	+ (DR) (T)	Rencüzoğullari et al. (2008)
Chromosomal aberrations	Human primary peripheral blood lymphocytes	50 µg/mL	NT	+ (DR) (T)	Rencüzoğullari et al. (2008)
	Chinese hamster lung fibroblasts	15 µg/mL[g]	+ (DR)	–	Sofuni et al. (1985)
	Chinese hamster lung fibroblasts	60 µg/mL	NT	–	Ishidate and Odashima (1977)
	Chinese hamster lung fibroblasts	125 µg/mL	NT	–	Ishidate et al. (1984)
	Chinese hamster lung (Don) cells	150 µg/mL	NT	–	Abe and Sasaki (1977)
DNA strand breaks	Mouse lymphoma L5178Y cells DNA alkaline elution assay	7.7 µg/mL	+ (DR)	– (T)	Garberg et al. (1988)
SCEs	Human primary peripheral blood lymphocytes	50 µg/mL[h]	NT	+ (DR) (T)	Rencüzoğullari et al. (2008)
	Chinese hamster lung (Don) cells	150 µg/mL	NT	±[i]	Abe and Sasaki (1977)
DNA repair	Human diploid lung fibroblasts (HSBP)	15 µg/mL	NT	–	Snyder and Matheson (1985)
Unscheduled DNA synthesis	Rat primary hepatocytes	15 µg/mL	NA	–	Hsia et al. (1983b, a)
	Rat primary hepatocytes	15 µg/mL	NA	–	Probst et al. (1981)
	Rat primary hepatocytes	15 µg/mL	NA	–	Williams et al. (1989); Williams (1978)
	Rat primary hepatocytes	150 µg/mL	NA	–	Brouns et al. (1979)
Cell transformation	Human diploid lung fibroblasts (WI-38) OR liver-derived cells (Chang)	250 µg/mL	–	NT	Purchase et al. (1978)
	Syrian hamster kidney cells BHK 21/cl 13	250 µg/mL	–	NT	
Mammalian systems in vivo					
Chromosomal aberrations	Rat, Sprague-Dawley, 5 males/dose, 20 inhalation exposures to biphenyl dust 7 hrs/d, 5 d/wk; bone marrow after 30 d	50 ppm	NA	–	Dow Chemical Co (1976)
Micronuclei	Mouse (CD-1), 6 males and females/dose, single oral gavage; bone marrow at 24 hrs	800 mg/kg	NA	–	Dow Chemical Co (2007)

Table C-3. Genotoxicity test results for biphenyl

Endpoint	Strain or test system	Dose/ concentration[a]	Results		Reference
			+S9	–S9	
DNA strand breaks	Mouse (ddY), 4 males/single oral dose; comet assay on stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow at 3 and 24 hrs	100 mg/kg	NA	+[j,k] (DR)	Sasaki et al. (2002)
DNA strand breaks	Mouse (CD-1), 4 males, single oral dose; comet assay on stomach, liver, kidney, bladder, lung, brain, and bone marrow at 3, 8, and 24 hrs	2,000 mg/kg	NA	+[k]	Sasaki et al. (1997)

[a]Lowest effective dose for positive results; highest dose for negative results.
[b]Tested range of S9 concentrations up to 100 µL/plate.
[c]80–85% survival at this dose. Positive results required test compound to be dissolved in dimethyl sulfoxide.
[d]Precipitation of test compound occurred at 100 µg/mL.
[e]Positive by two- to fourfold only at 10–20% total growth range; this was still within study guidelines for a positive result ($p \leq 0.001$).
[f]Positive ($p \leq 0.05$; pairwise t-test) at ≥30 µg/mL after a 24-hour incubation but only at 70 µg/mL after a 48-hour incubation
[g]No information on cytotoxicity provided.
[h]Positive ($p \leq 0.05$; pairwise t-test) at 70 µg/mL after a 24-hour incubation and ≥50 µg/mL after a 48-hour incubation.
[i]Positive results at 15, 75, and 150 µg/mL by pairwise t-test, but overall results considered negative by the authors due to lack of dose response.
[j]Positive ($p \leq 0.05$; Dunnett test) at 100 mg/kg in colon only; all other organs positive at 1,000 mg/kg.
[k]Positive results at 24 hours only.

– = negative result; + = positive result; ± = weakly positive or equivocal result; DR = dose-response observed; NA = not applicable; NT = not tested; T = cytotoxicity observed

Biphenyl metabolites. Table C-4 at the end of this section summarizes results from genotoxicity tests of several biphenyl metabolites, including 2-hydroxybiphenyl (also known as *o*-phenylphenol, or OPP), 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 (see(Balakrishnan et al., 2002; Kwok et al., 1999).

Limited evidence from bacterial assays specifically designed to detect oxidative DNA damage suggests that 2-hydroxybiphenyl may be mutagenic due to the formation of ROS resulting in the oxidation of DNA bases. This metabolite was positive in two bacterial strains developed to detect oxidative DNA damage: *S. typhimurium* strain TA102 and *E. coli* strain WP2*katEGsodAB* (Tani et al., 2007; Fujita et al., 1985). *S. typhimurium* strain TA102 was developed with an A:T base pair at the site of mutation and its sensitivity was increased by the addition of some 30 copies of a plasmid containing the mutant gene that are available for back mutation. This strain is sensitive to many oxidative mutagenic compounds, including quinones (Levin et al., 1982). *E. coli* strain WP2*katEGsodAB* is sensitive to ROS because this strain lacks

the detoxification enzymes, superoxide dismutase and catalase (Tani et al., 2007). In other bacterial mutagenicity tests, 2-hydroxybiphenyl showed mixed results. This metabolite was weakly mutagenic in a study of coded chemicals using *S. typhimurium* strains TA1535 without the addition of S9 from rat or hamster livers (Haworth et al., 1983). In this study, strain TA1535 showed a clear monotonic increase in mutagenicity up to 100 µg/plate; however, this response was slightly less than threefold of control levels, the criterion for considering a result positive in this strain. Another study using strain TA1535 for exposures up to 500 µg/plate did not replicate these results (Ishidate et al., 1984). Exposure of *B. subtilis* to 2-hydroxybiphenyl both with and without S9 in the recombinational repair assay yielded equivocal responses (Kojima and Hiraga, 1978; Hanada, 1977). In an in vivo mammalian cell assay, 2-hydroxybiphenyl did not induce chromosomal aberrations (without S9) in Chinese hamster lung fibroblasts (Ishidate et al., 1984).

In animal studies, 2-hydroxybiphenyl induced micronuclei (about threefold increase over controls) and increased cell proliferation (>200-fold increased incorporation of BrdU in DNA) in the bladder epithelium of male F344 rats exposed to 2% (20,000 ppm) in the diet for 2 weeks, without evidence of aneuploidy or polyploidy 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 sixfold 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 et al., 2002).

DNA damage was detected by the comet assay after 24-hour exposures in the urinary bladder of CD-1 mice administered single oral doses of 2,000 mg 2-hydroxybiphenyl/kg (Sasaki et al., 2002). This was the only organ to show evidence of DNA damage at 24 hours; after 3 hours of exposure, the colon (at 100 mg/kg doses), stomach, liver, kidney, and lung also showed signs of damage. The bone marrow and brain did not show DNA damage occurring at any timepoint. Another study of DNA strand breaks compared 2-hydroxybiphenyl with its metabolites, 2,5-dihydroxybiphenyl and phenylbenzoquinone. Using the alkaline elution assay, DNA strand breaks were 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 10,000 or 20,000 ppm, but not at 5,000 or 2,500 ppm (Morimoto et al., 1989).

Several investigators sought to determine whether 2-hydroxybiphenyl or its metabolites were capable of interacting directly with DNA. Using [^{32}P]-postlabeling to detect DNA adducts following 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, several DNA adducts in the skin were detected (Pathak and Roy, 1993). Similar adducts were formed in vitro when DNA was incubated with 2-hydroxybiphenyl (170 µg/mL) or 2,5-dihydroxybiphenyl (186 µg/mL) in the

presence of metabolic activation from rat skin homogenates (providing cytochrome P450 activation) 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 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. A 50–70-fold increase in the rate of cell division in the bladder epithelium of rats treated with 2% OPP in the 2-hydroxybiphenyl in the diet was also reported.

Bacterial mutation assays of the major biphenyl metabolite, 4-hydroxybiphenyl, were positive (threefold increase) in TA98 through 10 µg/plate using 20, 50, or 100 µL of S9; the response declined at higher concentrations, presumably due to toxicity. 4-Hydroxybiphenyl was marginally mutagenic in TA1535 (twofold increase), but only at the lowest concentration of S9 used (20 µL) (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).

Table C-4. Genotoxicity test results for biphenyl metabolites

Endpoint	Strain or test system	Dose/ concentration[a]	Results		Reference
			+S9	–S9	
2-Hydroxybiphenyl in vitro tests					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	200 µg/plate	–	–	Haworth et al. (1983)
	S. typhimurium TA1535	100 µg/plate	–	±	
	S. typhimurium TA98, TA100	100 µg/plate	–	–	Kojima and Hiraga (1978)
	S. typhimurium TA97a, TA102	10 µg/plate	+	–	Fujita et al. (1985)
	S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537, TA2637	500 µg/plate	–	–	Ishidate et al. (1984)
	E. coli WP2	100 µg/mL	–	–	Kojima and Hiraga (1978)
	E. coli WP2*katEGsodAB*, lacking catalase and superoxide dismutase	0.85 µg/mL	NT	+	Tani et al. (2007)

Table C-4. Genotoxicity test results for biphenyl metabolites

Endpoint	Strain or test system	Dose/ concentration[a]	Results		Reference
			+S9	–S9	
DNA recombination/ repair	*B. subtilis* rec assay H17 (rec^+), M45 (rec^-)	10,000 µg/plate	±	±	Kojima and Hiraga (1978)
Chromosomal aberrations	Chinese hamster lung fibroblasts	50 µg/mL	NT	–	Ishidate et al. (1984)
DNA adducts	Rat liver DNA [^{32}P]-post labeling method, in presence of skin homogenate or prostaglandin synthase activation systems	170 µg/mL	+[b]	NT	Pathak and Roy (1993)
2-Hydroxybiphenyl in vivo tests					
Micronuclei	Rat (F344) bladder epithelial cells, exposure in diet for 14 d, 5–9 males/group; significant cell proliferation was induced, but no ploidy changes were observed; cytotoxicity not measured	20,000 mg/kg	NA	+	Balakrishnan et al. (2002)
DNA strand breaks	Rat (F344) bladder epithelial cells, exposure in diet for 3–5 mos, 5–10 males and females/group; alkaline elution assay in bladder epithelial cells	10,000 mg/kg, sodium salt in diet	NA	+	Morimoto et al. (1989)
	Rat (F344), 6 males and females, 10-min exposures; alkaline elution assay in bladder epithelial cells	0.05% injected intravesically into bladder	NA	–[c]	
	Mouse (ddY), 4 males/single oral dose, comet assay				Sasaki et al. (2002)
	Colon (3 hrs)	100 mg/kg	NA	+	
	Stomach, colon, bladder, and lung (3 hrs)	1,000 mg/kg	NA	+	
	Stomach, colon, liver, kidney, and lung (3 hrs); bladder (24 hrs)	2,000 mg/kg	NA	+	
	Bone marrow and brain (3 and 24 hrs)	2,000 mg/kg	NA	–	
	Mouse (CD-1), 4 males, single oral dose, comet assay	2,000 mg/kg			Sasaki et al. (1997)
	Stomach (3 and 8 hrs), liver (3 hrs), kidney (3 and 8 hrs), bladder (8 and 24 hrs), and lung (3 hrs)		NA	+	
	Bone marrow and brain (3, 8, and 24 hrs)		NA	–	
DNA adducts	Mouse (CD-1), 6 females/dose; [^{32}P]-postlabeling of DNA isolated from skin	10 or 20 mg applied to skin	NA	+	Pathak and Roy (1993)

Table C-4. Genotoxicity test results for biphenyl metabolites

Endpoint	Strain or test system	Dose/ concentration[a]	Results		Reference
			+S9	–S9	
	Rat (F344) bladder epithelial cells, 20–40/group, in diet for 13 wks [^{32}P]-postlabeling method; cytotoxicity and cell proliferation observed ≥8,000 mg/kg	12,500 mg/kg	NA	–	Smith et al. (1998)
	Rat (F344) bladder epithelial cells, 4 males/group, single dose by gavage, labeled with [^{14}C]-2-hydroxy-biphenyl (uniformly labeled in phenol ring); protein adducts were observed	1,000 mg/kg	NA	–	Kwok et al. (1999)
4-Hydroxybiphenyl in vitro tests					
Reverse mutation	*S. typhimurium* TA98,	10 µg/plate	+	NT	Narbonne et al. (1987)
	TA1535		±	NT	
2,5-Dihydroxybiphenyl in vitro or in vivo tests					
DNA strand breaks	Human DNA fragments from plasmid pbcNI measured by gel electrophoresis	18.6 µg/mL	NT	+[d]	Inoue et al. (1990)
	Rat (F344), 6 males and females, 10-min exposures, alkaline elution assay in bladder epithelial cells	0.05% injected intravesically into bladder	NA	–[c]	Morimoto et al. (1989)
DNA adducts	Rat liver DNA; [^{32}P]-post labeling method, in presence of skin homogenate or prostaglandin synthase activation systems	186 µg/mL	+[b]	NT	Pathak and Roy (1993)
	Mouse (CD-1), 6 females/dose; [^{32}P]-postlabeling of DNA isolated from skin	5 mg applied to skin	NA	+	Pathak and Roy (1993)

[a]Lowest effective dose for positive results; highest dose for negative results.
[b]Skin homogenate used as source of cytochrome P450 activation system.
[c]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%.
[d]Positive response only in the presence of Cu(II).

± = weakly positive or equivocal result; DR = dose response observed; NA = not applicable; NT = not tested

Synthesis of genotoxicity evidence for biphenyl and its metabolites. A review of the evidence for the genotoxic potential of biphenyl suggests that there may be some ability of this compound to induce genetic damage. Although bacterial mutagenicity assays are uniformly negative, even with metabolic activation, several in vitro assays were able to detect weak evidence of mutagenicity with activation (Glatt et al., 1992; Wangenheim and Bolcsfoldi, 1988). Indications of the ability to induce chromosomal aberrations were also observed (Sofuni et al., 1985), although this was accompanied by cytotoxicity in one study (Rencüzoğullari et al., 2008). In addition, evidence of DNA strand breaks was observed in mice in several organs, including

the stomach, blood, liver, bone marrow, kidney, bladder, lung, and brain (Sasaki et al., 2002; Sasaki et al., 1997). Micronuclei were observed in primary human lymphocytes (Rencüzoğullari et al., 2008), but were not found in another study in mouse bone marrow (Dow Chemical Co, 2007), and chromosomal aberrations were not observed following inhalation exposures in rats (Dow Chemical Co, 1976).

There are indications that the metabolites of biphenyl may be more genotoxic than the parent compound. Genotoxicity results for the major metabolite, 4-hydroxybiphenyl, and a minor metabolite, 2-hydroxybiphenyl (i.e., *o*-phenylphenol, or OPP), can be found in Table C-4. Metabolism of 2-hydroxybiphenyl may induce oxidative DNA damage resulting from redox cycling between 2,5-dihydroxybiphenyl and phenylbenzoquinone (Sasaki et al., 2002; Sasaki et al., 1997; Pathak and Roy, 1993; Morimoto et al., 1989). Limited evidence for this can be found in positive results in two bacterial strains developed to be sensitive to oxidative DNA damage (Tani et al., 2007; Fujita et al., 1985).

Other investigations in vivo appear to corroborate these findings. Balakrishnan et al. (2002) reported that 2-hydroxybiphenyl induced micronuclei and increased cell proliferation in the bladder epithelium of male F344 rats. The mechanism of 2-hydroxybiphenyl-induced micronuclei is not understood, but, as discussed by Balakrishnan 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.

Finding evidence that biphenyl can react directly with DNA when metabolized would provide evidence that oxidative damage and subsequent cytotoxicity and regenerative cell proliferation may not be solely responsible for findings of genotoxicity; these processes are not mutually exclusive. No investigations of the DNA binding potential of biphenyl either in vivo or in activated systems have been reported, but several studies reported on tests performed specifically on the metabolites. One such study, Pathak and Roy (1993), reported finding DNA adducts with rat DNA in vitro and from mouse skin treated with 2-hydroxybiphenyl and 2,5-dihydroxybiphenyl in vivo. However, these results could not be reproduced by other groups specifically looking at the rat bladder, the target organ for carcinogenicity, following oral exposures (Kwok et al., 1999; Smith et al., 1998). Although topical application to mouse skin does not represent the primary route of exposure or target organ for biphenyl, such contradictory reports do not rule out the possibility that biphenyl metabolites may be able, in some circumstances, to bind DNA. However, the Smith and Kwok studies also reported significant cytotoxicity and cell proliferation, providing more evidence of a secondary source for DNA damage following biphenyl exposures.

Sasaki et al. (2002) and Sasaki et al. (1997), who reported DNA strand breaks in several mouse organs following oral exposure to biphenyl, also reported similar damage following oral

exposure to 2-hydroxybiphenyl. However, the timing and the pattern of organs affected was slightly different. The DNA damage was only detected early (3 hours) after initial exposure in several organs (stomach, liver, kidney, and lung) and began to disappear 8 hours after exposure. The exception was the bladder, in which damage was first detected at 8 hours and persisted 24 hours after exposure. A reasonable explanation for these results is that DNA damage was repaired over time in most organs, but was increased in the bladder where this compound becomes concentrated due to its excretion in the urine.

To summarize, it is unknown if reports of DNA damage following exposures to biphenyl are caused by a direct reaction with DNA or by indirect damage from cytotoxicity, or ROS generated from redox cycling of hydroquinone metabolites, or some combination of these mechanisms. Biphenyl in an activated system was not investigated for its ability to form DNA-reactive metabolites, but in studies of DNA adduct formation using the metabolites, most were negative (Kwok et al., 1999; Smith et al., 1998), save for one study of very high doses applied to skin (Pathak and Roy, 1993). However, several reports outlined above indicate that genetic damage induced by biphenyl or its metabolites often occurred only after very high doses that were accompanied by decreased cell survival or concurrent with redox cycling following metabolism of 2-hydroxybiphenyl, a minor metabolite. One study that directly tested the mutagenicity of the major metabolite, 4-hydroxyquinone, in the Ames assay was positive (Narbonne et al., 1987), but no other investigations of this metabolite were located. In addition, since the relative production of these metabolites is unknown in humans, damage occurring due to 2-hydroxybiphenyl may still be important for understanding genotoxic risk following biphenyl exposures. In summary, there is not enough evidence to conclude that biphenyl is mutagenic or can react with DNA, but the overall implication is that most indications of genotoxicity following biphenyl exposures are likely to be secondary responses resulting from oxidative damage and cytotoxicity.

APPENDIX D. 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 D-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 D-2. The dataset for incidence of fetuses with missing or unossified sternebrae from Wistar rat dams administered biphenyl by gavage on GDs 6–15 (Khera et al., 1979) is shown in Table D-3.

Table D-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**
Calculated dose (mg/kg-d)	**0**	**36.4**	**110**	**378**	**0**	**42.7**	**128**	**438**
Effect								
Renal pelvis								
Nodular transitional cell hyperplasia	0	1	1	21	0	0	1	12
Simple transitional cell hyperplasia	6	8	5	19	3	5	12	25
Mineralization	9	6	10	18	12	12	18	27
Other kidney effects								
Hemosiderin deposit[a]	0	0	0	0	4	8	22	25
Papillary mineralization	9	9	14	23	2	6	3	12
Bladder								
Combined transitional cell hyperplasia[b]	0	0	0	45	1	0	1	10

[a]Male data for incidences of hemosiderin deposits not selected for quantitative analysis.
[b]Female data for incidences of combined transitional cell hyperplasia not selected for quantitative analysis.

Source: Umeda et al. (2002).

Table D-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	22.9 ± 2.7
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	32.4 ± 3.6
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	26
Clinical chemistry parameter	n = 28	n = 20	n = 22	n = 31
AST (IU/L)	75 ± 27	120 ± 110	211 ± 373	325 ± 448
ALT (IU/L)	32 ± 18	56 ± 46	134 ± 231	206 ± 280
AP (IU/L)	242 ± 90	256 ± 121	428 ± 499	556 ± 228
LDH (IU/L)	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161
BUN (mg/dL)	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7
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	25.5 ± 3.0

Source: Umeda et al. (2005).

Table D-3. BMD modeling dataset for incidence of fetuses with missing or unossified sternebrae from Wistar rat dams administered biphenyl by gavage on GDs 6–15

Effect	Dose (mg/kg-d)			
	0	**125**	**250**	**500**
Fetuses with missing or unossified sternebrae[a]/animals examined (number of litters examined)	4/176 (16)	3/236 (20)	4/213 (18)	16/199 (18)

[a]Data from the 1,000 mg/kg-day dose group were not included because of frank maternal toxicity at that dose.

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

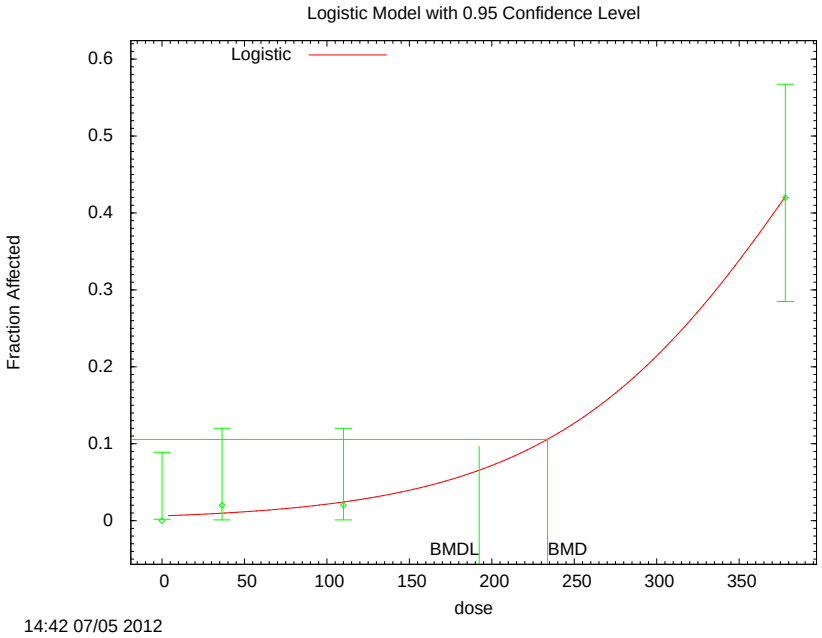
Tables D-4 through D-24. Each table of modeled results for a particular effect is followed by the information from the output file of the best-fitting model for that effect.

Table D-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]	Residual with the largest absolute value	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[c]	**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 (2-degree)[d]	0.39	-0.99	93.56	109.09	64.15	162.37	116.56
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.

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)
```

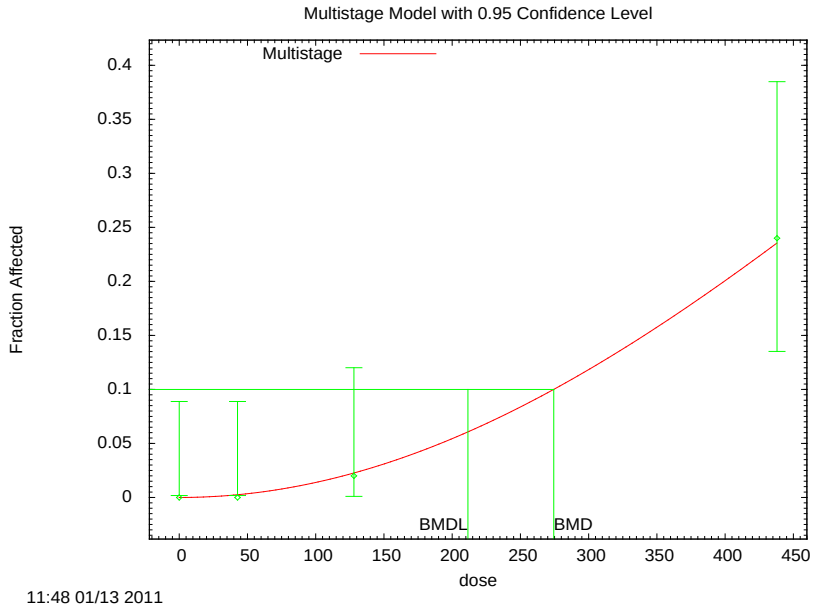
```
         Input Data File:
C:\USEPA\BMDS212\Data\Biphenyl\RenalNodularTransCellHyperPlasia_Umeda2002\-Umeda 2002-Renal
Nodular Transitional Cell Hyperplasia F Rat-Logistic-10%.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\Biphenyl\RenalNodularTransCellHyperPlasia_Umeda2002\-Umeda 2002-Renal
Nodular Transitional Cell Hyperplasia F Rat-Logistic-10%.plt
                                                    Thu Jul 05 14:42:08 2012
 ========================================================================
 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 =     -4.37631
                         slope =    0.0106422

          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        -0.95
     slope        -0.95            1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     intercept         -5.07619         0.879668            -6.8003            -3.35207
         slope        0.0125723       0.00249823          0.00767588           0.0174688

                      Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -43.8185         4
   Fitted model        -44.3579         2       1.07873      2          0.5831
  Reduced model        -71.3686         1       55.1002      3         <.0001
          AIC:          92.7157

                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0062         0.310     0.000          50       -0.559
   36.4000     0.0098         0.489     1.000          50        0.735
  110.0000     0.0243         1.214     1.000          50       -0.197
  378.0000     0.4197        20.987    21.000          50        0.004
 Chi^2 = 0.89      d.f. = 2        P-value = 0.6403

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
            BMD =        233.809
           BMDL =        192.347
```

Table D-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]	Residual with the largest absolute value	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.

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

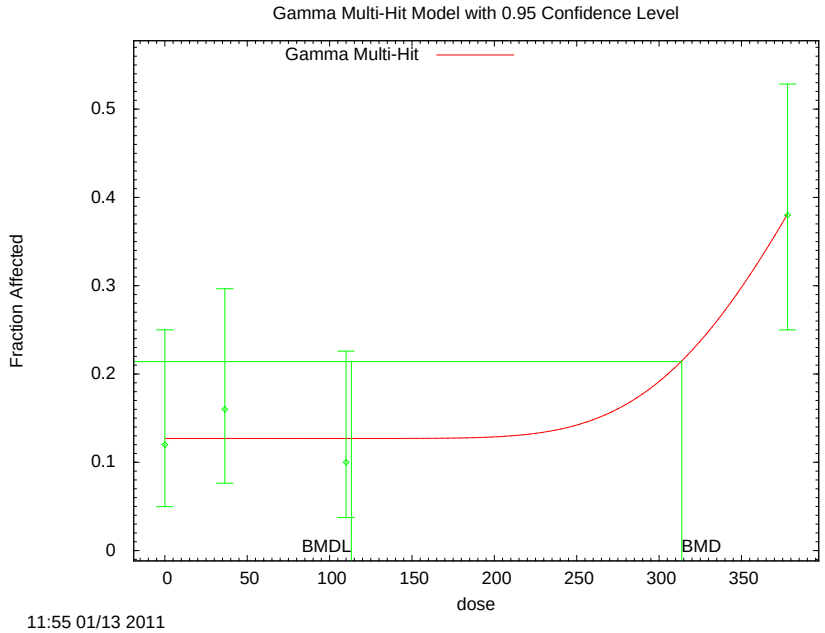
```
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1-beta2*dose^2)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing 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 D-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]	Residual with the largest absolute value	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.

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

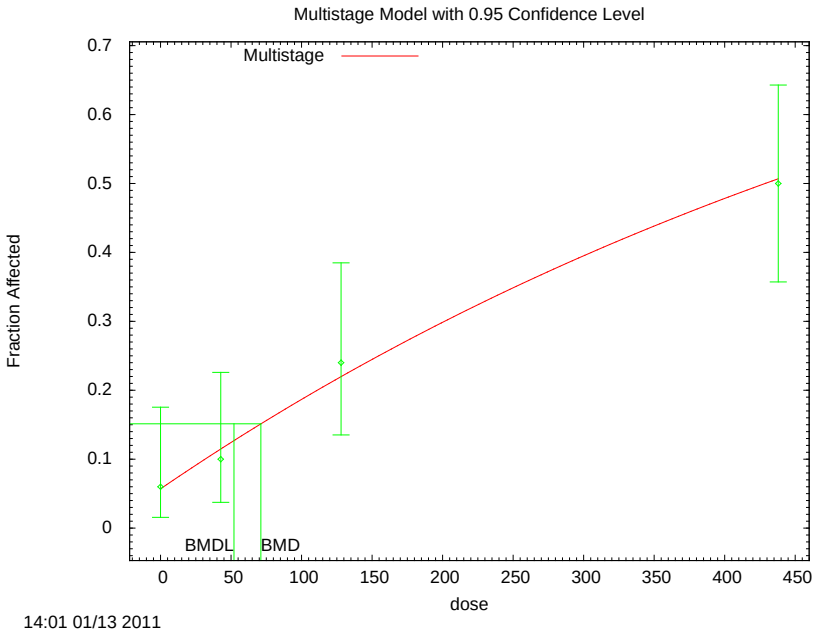
Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 313.755
BMDL = 113.219
~~~~~~

Table D-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]	Residual with the largest absolute value	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.

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

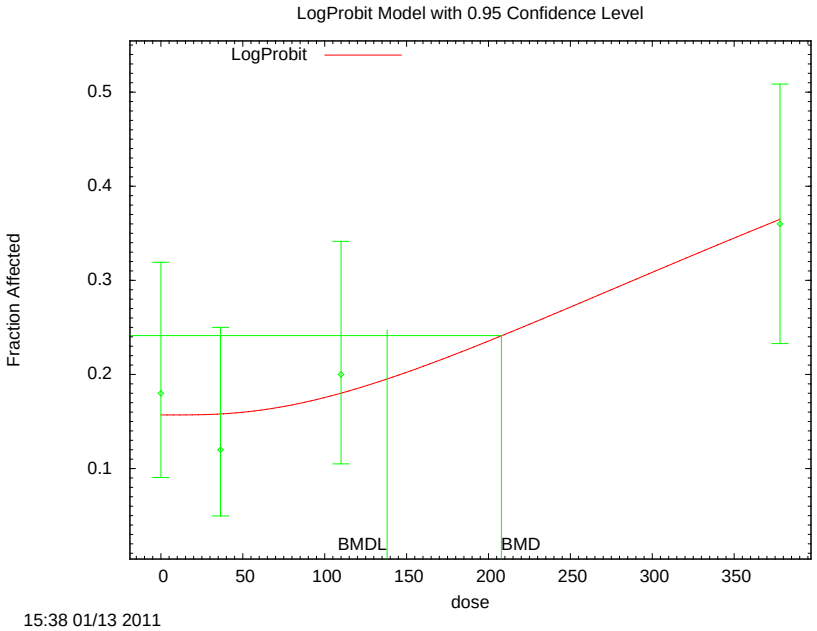
```
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing values = 0
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 D-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]	Residual with the largest absolute value	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.

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

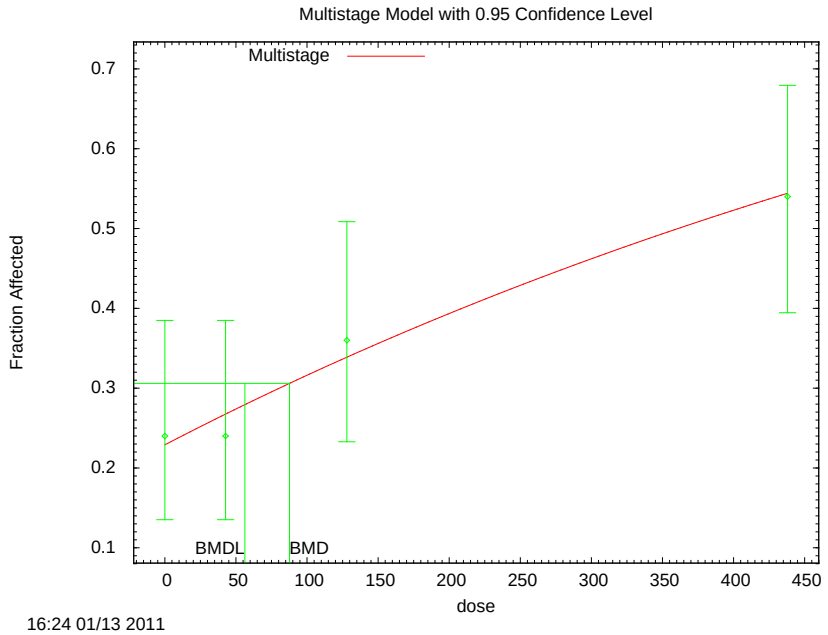
```
The form of the probability function is: P[response] = Background + (1-Background) *
CumNorm(Intercept+Slope*Log(Dose)), where CumNorm(.) is the cumulative normal distribution
function
Dependent variable = incidence
Independent variable = dose
Slope parameter is restricted as slope >= 1
Total number 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 D-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]	Residual with the largest absolute value	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.

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

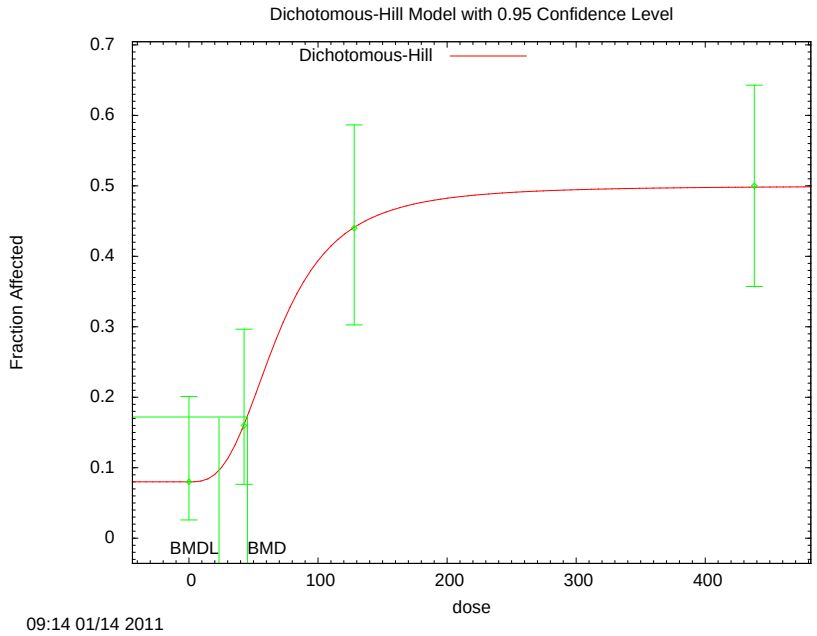
```
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing values = 0
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 D-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]	Residual with the largest absolute value	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).

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
 =================================================================
```

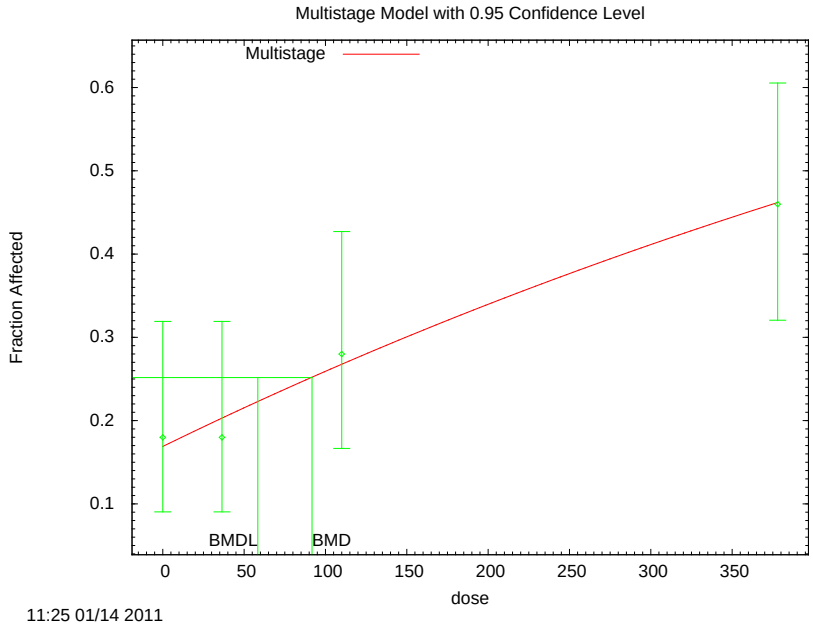
```
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = v*g +(v-v*g)/[1+EXP(-intercept-
slope*Log(dose))] where: 0 <= g < 1, 0 < v <= 1v is the maximum probability of response predicted
by the model, and v*g 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 D-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]	Residual with the largest absolute value	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.

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

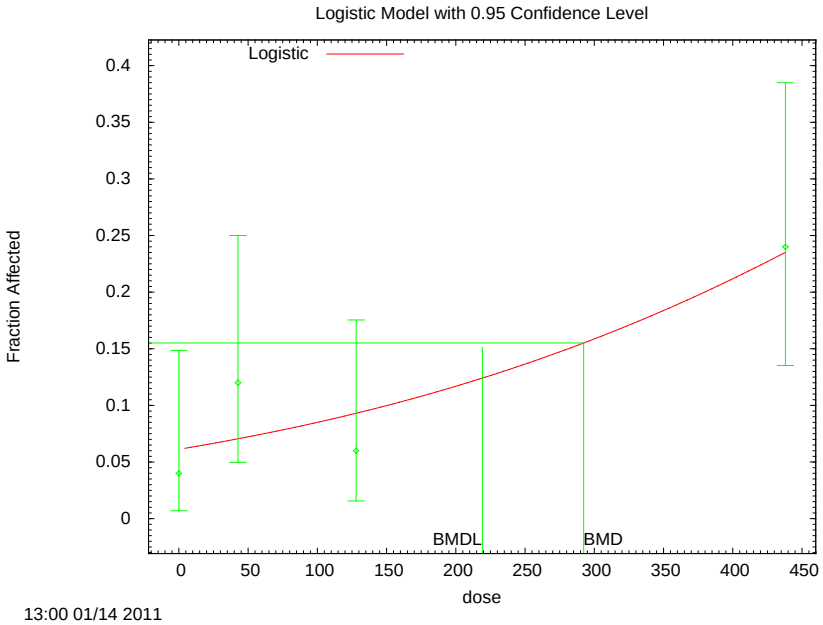
```
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-
beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = incidence
Independent variable = dose
Total number of observations = 4
Total number of records with missing values = 0
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 D-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]	Residual with the largest absolute value	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.

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)]
```

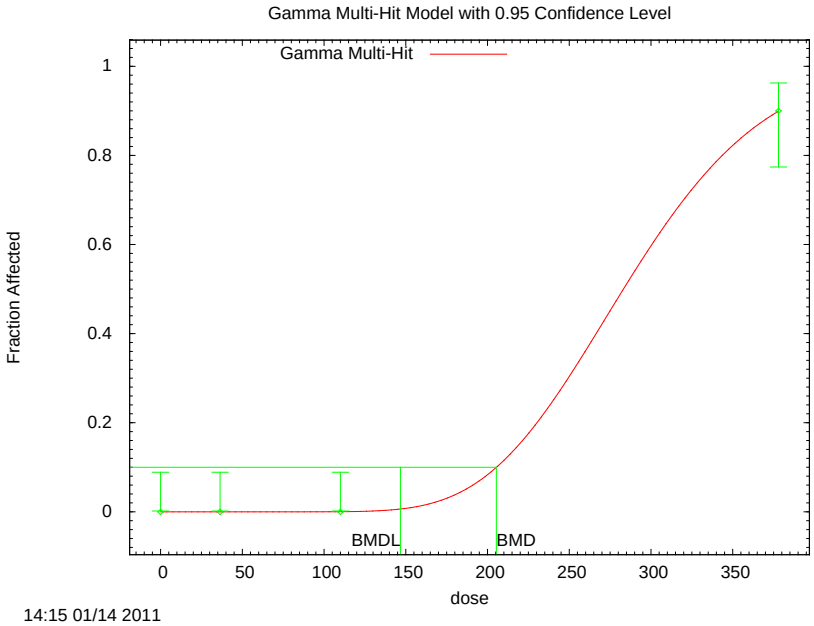
```
Dependent variable = incidence
Independent variable = dose
Slope parameter is not restricted
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set 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 D-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]	Residual with the largest absolute value	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.

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

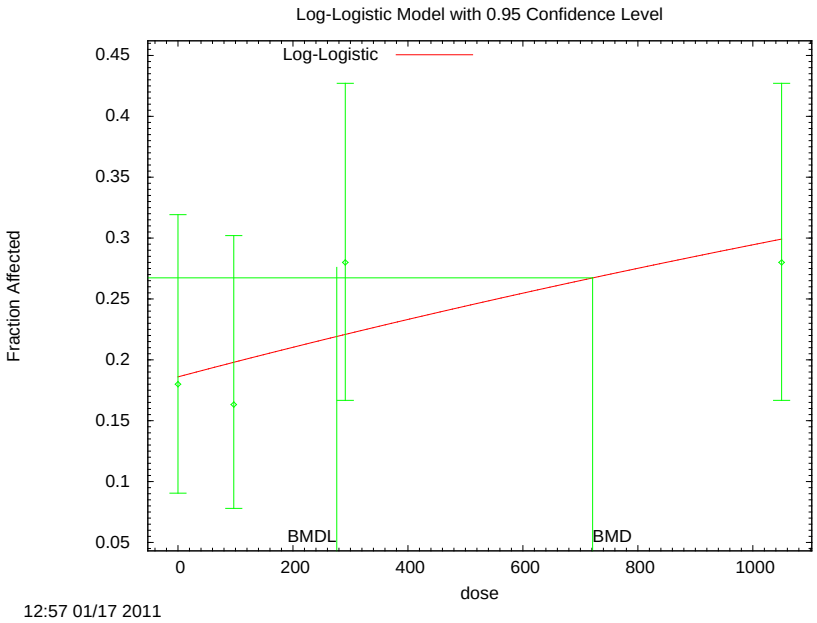
Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 205.404
BMDL = 146.733
~~~~~~

Table D-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]	Residual with the largest absolute value	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.

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
```

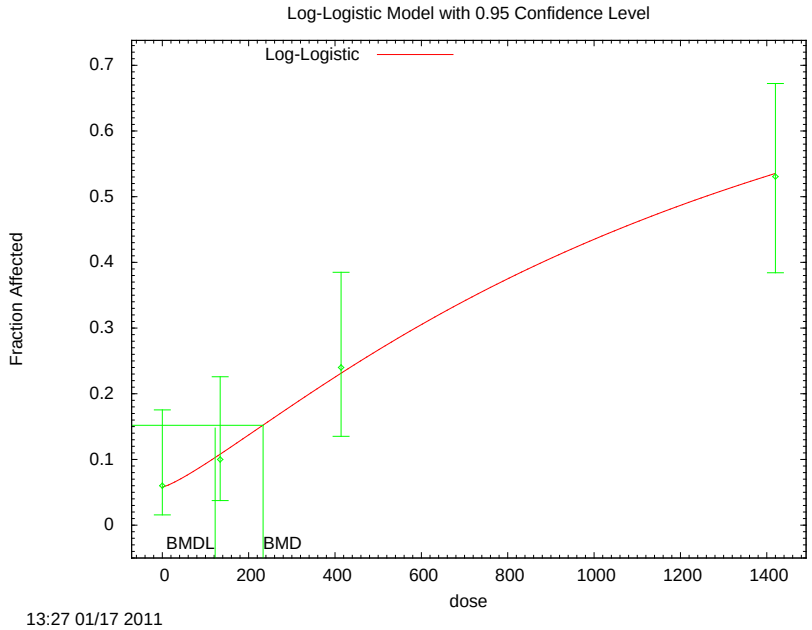
```
Independent variable = dose
Slope parameter is restricted as slope >= 1
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
User has 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 D-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]	Residual with the largest absolute value	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.

Source: Umeda et al. 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 D-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]	Residual with the largest absolute value	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.

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.

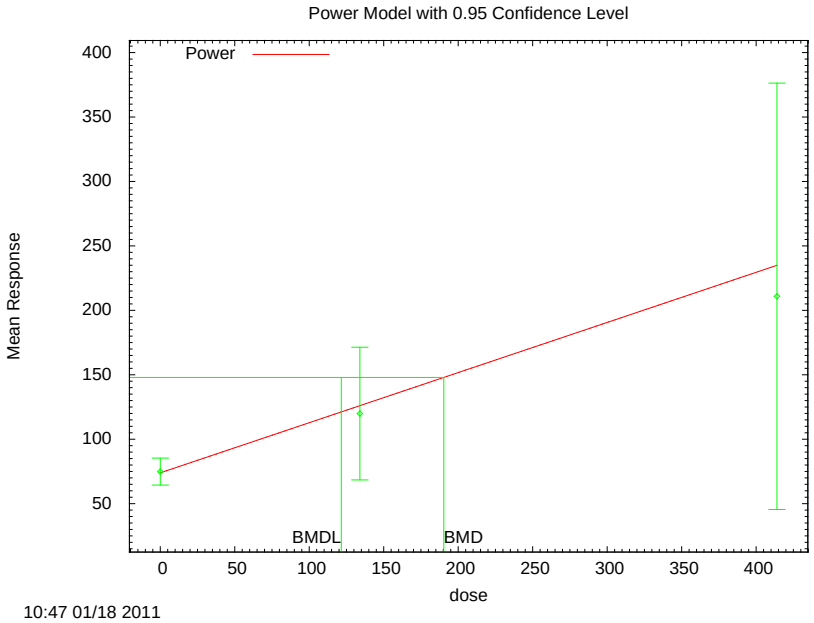
Table D-17. BMD modeling results for serum AST activity in female BDF_1 mice exposed to biphenyl in the diet for 2 years

Model	Goodness of fit				Benchmark result (mg/kg-d)			
	Variance model *p*-value[a]	Means model *p*-value[a]	Residual with the largest absolute value	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.

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 100% 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 D-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]	Residual with the largest absolute value	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.

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 D-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]	Residual with the largest absolute value	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.

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 D-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]	Residual with the largest absolute value	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.

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 fit both the variance and means; however, BMDs at the selected BMRs, both 1SD and 1RD, were higher than the highest observed dose in the model. Therefore, modeling was not adequate or suitable for the data on BUN level in male mice exposed to biphenyl in the diet for 2 years.

Table D-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]	Residual with the largest absolute value	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.

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 D-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]	Residual with the largest absolute value	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.
CF = computation failed; NA = not applicable

Source: Umeda et al. (2005).

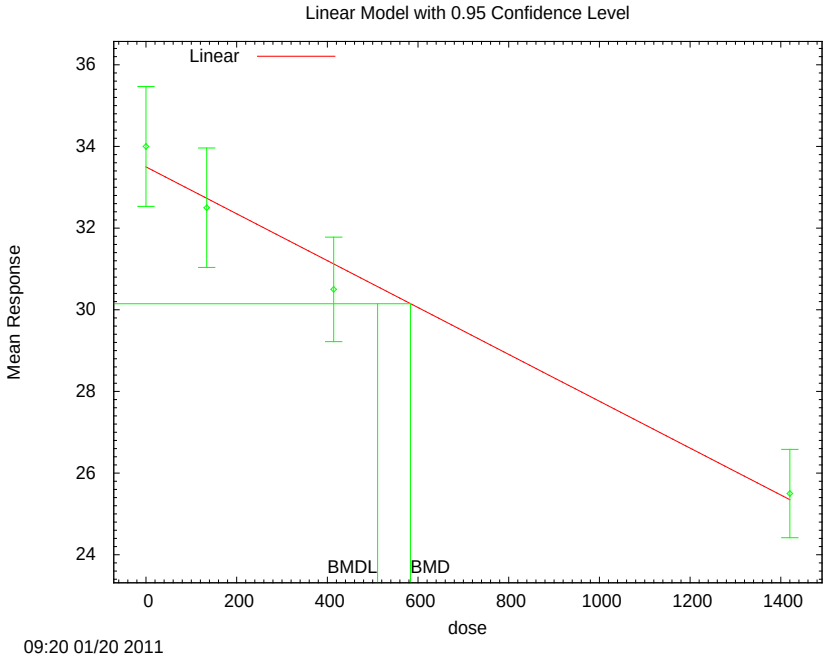
The constant variance models did not fit either the variance data or the means data. The nonconstant variance models failed to fit the variance data. When the data from the highest dose group were dropped, the constant variance models did not fit either the variance data or the means data. The nonconstant 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 D-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]	Residual with the largest absolute value	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.

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

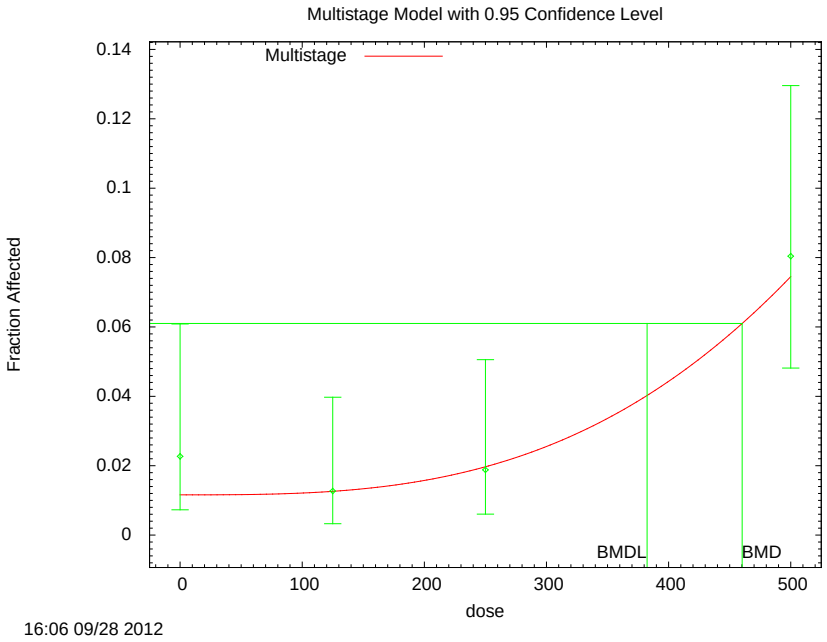
Table D-24. Summary of BMD modeling results for fetal incidence of missing or unossified sternebrae from Wistar rat dams administered biphenyl by gavage on GDs 6–15 (the highest dose was not included because of maternal toxicity)

Model	Goodness of fit			Benchmark result (mg/kg-d)			
	χ^2 *p*-value[a]	Residual with the largest absolute value	AIC	BMD_5	$BMDL_5$	BMD_{10}	$BMDL_{10}$
BMDS modeling with sample size = total number of fetuses examined							
Gamma[b]	0.44	0.58	227.97	472.48	386.02	554.43	497.84
Logistic	0.18	1.46	228.47	447.48	371.37	614.46	502.98
Log-Logistic[b]	0.44	0.58	227.97	476.11	388.23	545.44	498.10
Log-Probit[b]	0.44	0.59	227.97	469.56	379.56	562.13	497.60
Multistage (3-degree)[c,d]	**0.37**	**1.38**	**204.28**	**460.22**	**382.38**	**585.02**	**502.28**
Probit	0.15	1.48	228.89	448.57	361.27	645.350	510.69
Weibull[b]	0.44	0.58	227.97	476.62	389.54	543.82	498.17
BMDS modeling with sample size = total number of litters examined							
Gamma[b]	0.82	0.17	25.67	473.31	177.26	553.58	349.07
Logistic	0.86	0.42	23.89	447.38	264.21	615.71	379.80
Log-Logistic[b,d]	**0.82**	**0.17**	**25.67**	**476.95**	**173.39**	**544.46**	**348.52**
Log-Probit[b]	0.82	0.17	25.67	470.45	below zero	561.16	340.36
Multistage (2-degree)[c]	CF	CF	10.27	542.30	243.88	503.58	260.59
Probit	0.85	0.43	23.93	448.31	248.01	646.43	366.98
Weibull[b]	0.82	0.17	25.67	477.45	177.25	542.86	350.99

[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.

CF = computation failed

Source: Khera et al. (1979).



BMD and BMDL indicated are associated with an extra risk of 5%, with t**otal fetuses** examined in each dose group as the sample size and are in units of mg/kg-day.

```
=====================================================================
          Multistage Model. (Version: 3.2;  Date: 05/26/2010)
          Input Data File:
C:\USEPA\BMDS212\Data\Biphenyl\Sternebrae_Khera1979\Sternebrae_fetal%_fetalN\mst_Sternebrae_fetal
%_fetalN_M3.(d)
          Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\Biphenyl\Sternebrae_Khera1979\Sternebrae_fetal%_fetalN\mst_Sternebrae_fetal
%_fetalN_M3.plt
                                                    Thu Sep 27 16:41:03 2012
 =====================================================================
 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 = fetal_pct
   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 =     0.011904
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 5.52452e-010

            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Beta(1)    -Beta(2)
                  have been estimated at a boundary point, or have been specified by the user,
                  and do not appear in the correlation matrix )
```

	Background	Beta(3)
Background	1	-0.51
Beta(3)	-0.51	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0115907	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	5.26214e-010	*	*	*

* - Indicates that this value is not calculated.

Warning: Likelihood for the fitted model larger than the Likelihood for the full model.
Error in computing chi-square; returning 2

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-110.686	4			
Fitted model	-100.14	2	-21.0916	2	2
Reduced model	-118.836	1	16.2989	3	0.0009847

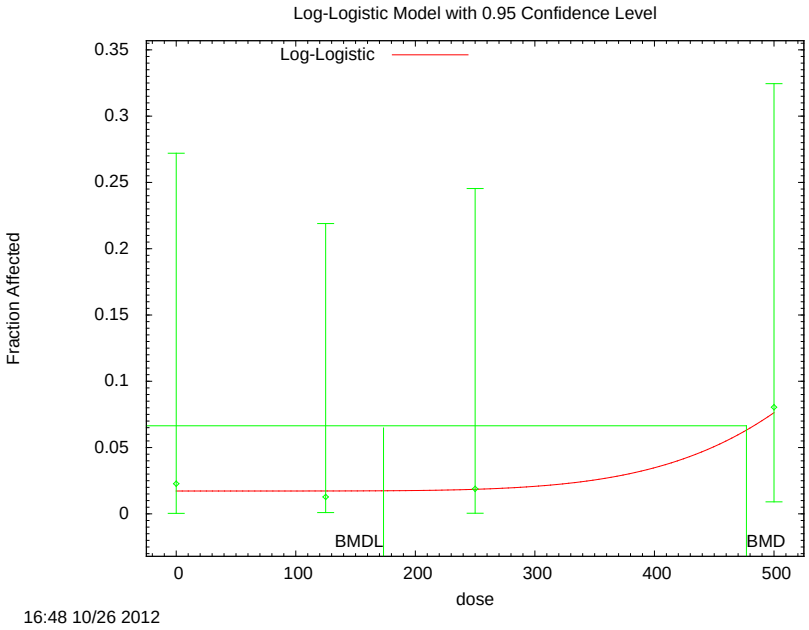
AIC: 204.281

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0116	2.040	3.995	176	1.377
125.0000	0.0126	2.975	2.997	236	0.013
250.0000	0.0197	4.193	4.004	213	-0.093
500.0000	0.0745	14.828	16.000	199	0.316

Chi^2 = 2.00 d.f. = 2 P-value = 0.3670

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 460.221
BMDL = 382.382
BMDU = 576.027
Taken together, (382.382, 576.027) is a 90 % two-sided confidence interval for the BMD



BMD and BMDL indicated are associated with an extra risk of 5%, with **total litters** examined in each dose group as the sample size and are in units of mg/kg-day.

```
 ======================================================================
         Logistic Model. (Version: 2.13; Date: 10/28/2009)
         Input Data File:
C:\USEPA\BMDS212\Data\Biphenyl\Sternebrae_Khera1979\Sternebrae_Fetal%_LitterN\lnl_Sternebrae_Feta
l%_LitterN_LogLogistic.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\Biphenyl\Sternebrae_Khera1979\Sternebrae_Fetal%_LitterN\lnl_Sternebrae_Feta
l%_LitterN_LogLogistic.plt
                                                    Thu Sep 27 15:46:15 2012
 ======================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = FetalPct
   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 =       0.0227
                     intercept =     -8.88585
                         slope =            1

          Asymptotic Correlation Matrix of Parameter Estimates
            background    intercept        slope
background           1        -0.54         0.54
 intercept       -0.54            1           -1
     slope        0.54           -1            1

                              Parameter Estimates
                                                    95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background        0.0172336            *                 *                   *
     intercept         -37.7537            *                 *                   *
         slope          5.64407            *                 *                   *
```

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-9.81066	4			
Fitted model	-9.83702	3	0.0527153	1	0.8184
Reduced model	-10.5318	1	1.44237	3	0.6956
AIC:	25.674				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0172	0.276	0.363	16	0.168
125.0000	0.0173	0.345	0.254	20	-0.157
250.0000	0.0186	0.334	0.338	18	0.007
500.0000	0.0804	1.447	1.447	18	-0.000

Chi^2 = 0.05 d.f. = 1 P-value = 0.8183

Benchmark Dose Computation

Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 476.945
BMDL = 173.393

APPENDIX E. 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 E-1.

Table E-1. Incidences of liver adenomas or carcinomas 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	3/48[a]	8/50	16/49[a]	14/48[a]

[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.

Source: Umeda et al. (2005).

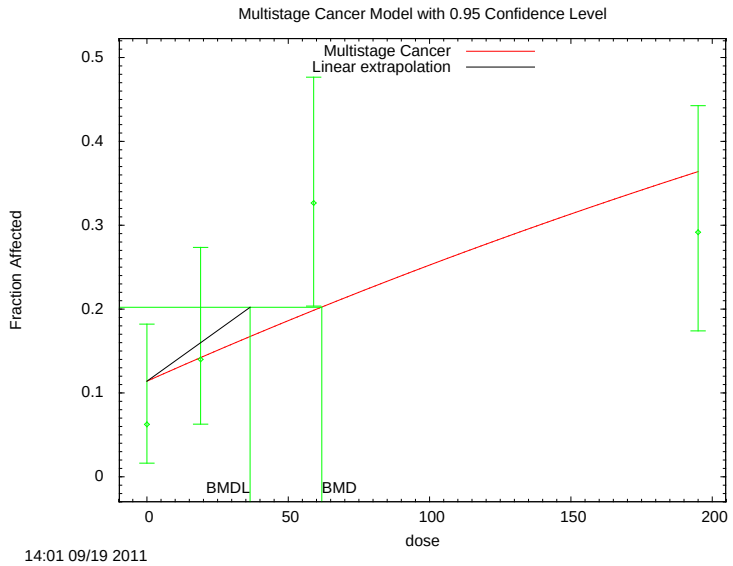
Summaries of the BMDs, BMDLs, and derived oral slope factors for the modeled mouse data are presented in Table E-2, followed by the plot and model output file from the best-fitting model. The incidence of liver tumors exhibited a plateau in animals in two highest dose groups. To better estimate responses 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 E-2. Model predictions for liver tumors (adenomas or carcinomas) in female BDF_1 mice exposed to biphenyl in the diet for 2 years

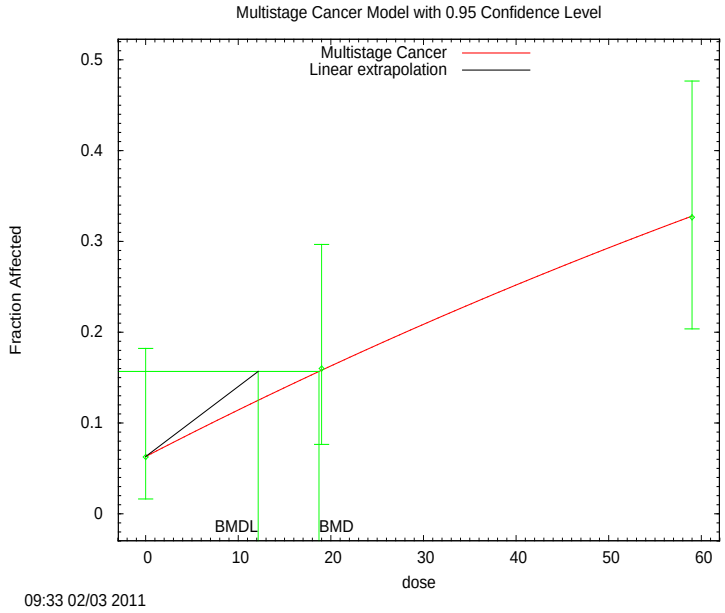
Model	Goodness of fit			Benchmark result (mg/kg-d)		
	χ^2 *p*-value[a]	Residual with the largest absolute value	AIC	BMD_{HED10}	$BMDL_{HED10}$	Cancer slope factor (risk per mg/kg-d)
All doses						
Multistage (1-, 2-, 3-degree)[b], Gamma[c], Weibull[c]	0.03	2.14	197.37	64.76	37.29	3×10^{-3}
Logistic	0.01	2.31	198.96	104.91	71.27	1×10^{-3}
Log-Logistic[c]	0.04	1.97	196.62	50.68	26.80	4×10^{-3}
Log-Probit[c]	0.005	2.58	201.06	128.52	74.43	1×10^{-3}
Probit	0.01	2.30	198.80	100.16	67.23	1×10^{-3}
Highest dose dropped						
Multistage (1-degree)[b,d]	**0.96**	**0.04**	**132.32**	**18.72**	**12.15**	**8×10^{-3}**
Multistage (2-degree)[b]	0.96	0.04	132.32	18.72	12.15	8×10^{-3}

[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.

Source: Umeda et al. (2005).



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.



The BMDS graph of multistage (1-degree) model with the highest dose 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
```

The urinary bladder tumor dataset from Umeda et al. (2002) for which dose-response modeling was performed is shown in Table E-3.

Table E-3. Incidences of urinary bladder transitional cell papilloma or carcinoma in male F344 rats fed diets containing biphenyl for 2 years

Biphenyl dietary concentration (ppm)	**0**	**500**	**1,500**	**4,500**
Reported dose (mg/kg-d)	**0**	**36.4**	**110**	**378**
HED (mg/kg-d)	**0**	**10**	**30**	**101**
Tumor incidence				
Papilloma or carcinoma	0/50	0/50	0/50	31/49[a]

[a]One high-dose male rat was excluded from denominators because of death prior to week 52. It is assumed that this rat did not have tumors 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.

Source: Umeda et al. (2002).

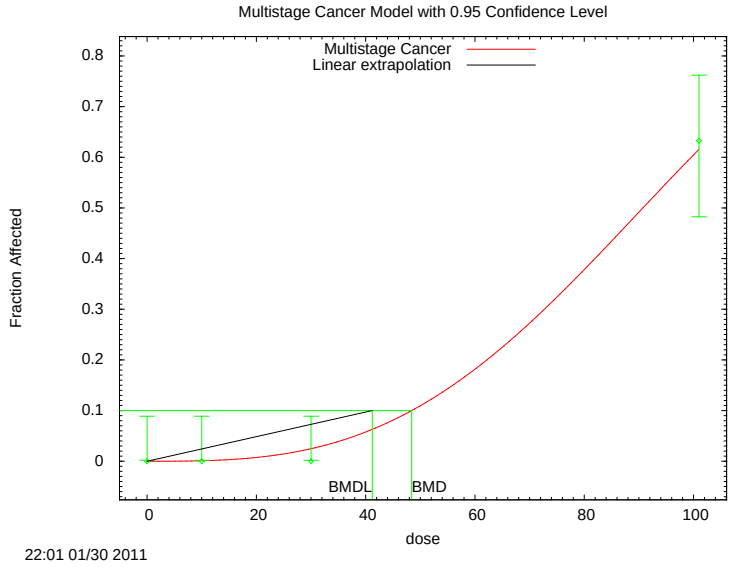
Summaries of the BMDs, BMDLs, and a derived oral slope factors for the modeled mouse data are presented in Table E-4, followed by the plot and model output file from the best-fitting model.

Table E-4. Model predictions for urinary bladder tumors (papillomas or carcinomas) 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]	Residual with the largest absolute value	AIC	BMD_{HED10}	$BMDL_{HED10}$	Cancer slope factor (risk per mg/kg-d)
Multistage (1-degree)[b]	0.0002	-3.120	96.71	17.77	13.34	8×10^{-3}
Multistage (2-degree)[b]	0.1713	-1.980	75.50	35.44	30.44	3×10^{-3}
Multistage (3-degree)[b]	**0.7113**	**-1.126**	**69.10**	**48.42**	**41.21**	**2×10^{-3}**

[a]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[b]Betas restricted to ≥0.

Source: Umeda et al. (2002).



The BMDS graph of multistage (3-degree) model.
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:/USEPA/IRIS/biphenyl/2011/rat/bladdertumor/revised/msc_bladtumMrev_MS_3.(d)
         Gnuplot Plotting File:
C:/USEPA/IRIS/biphenyl/2011/rat/bladdertumor/revised/msc_bladtumMrev_MS_3.plt
                                                  Sun Jan 30 22:01:35 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: 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
                        Beta(1) =           0
                        Beta(2) =           0
                        Beta(3) = 9.80294e-007
           Asymptotic Correlation Matrix of Parameter Estimates
         ( *** The model parameter(s)  -Background    -Beta(1)    -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(3)

   Beta(3)           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)               0            *                *                   *
        Beta(3)     9.27909e-007           *                *                   *
* - Indicates that this value is not calculated.

                     Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -32.2189         4
   Fitted model        -33.5483         1       2.65884      3          0.4473
  Reduced model        -86.0881         1       107.738      3         <.0001
           AIC:         69.0966

                               Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          50        0.000
   10.0000     0.0009         0.046     0.000          50       -0.215
   30.0000     0.0247         1.237     0.000          50       -1.126
  101.0000     0.6156        30.164    31.000          49        0.246
 Chi^2 = 1.38      d.f. = 3        P-value = 0.7113

   Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        48.4236
            BMDL =        41.2077
            BMDU =         53.891
Taken together, (41.2077, 53.891 ) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00242673
```

APPENDIX F. DOCUMENTATION OF IMPLEMENTATION OF THE 2011 NATIONAL RESEARCH COUNCIL RECOMMENDATIONS

Background: On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law[11]. The report language included direction to EPA for the Integrated Risk Information System (IRIS) Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde[12]. The report language included the following:

> The Agency shall incorporate, as appropriate, based on chemical-specific data sets and biological effects, the recommendations of Chapter 7 of the National Research Council's Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde into the IRIS process…For draft assessments released in fiscal year 2012, the Agency shall include documentation describing how the Chapter 7 recommendations of the National Academy of Sciences (NAS) have been implemented or addressed, including an explanation for why certain recommendations were not incorporated.

The NRC's recommendations, provided in Chapter 7 of the review report, offered suggestions to EPA for improving the development of IRIS assessments. Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in the tables below. Where necessary, the documentation includes an explanation for why certain recommendations were not incorporated.

The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that, "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

The IRIS biphenyl assessment is in Phase 1 of implementation, which focuses on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focuses on assessments near the end of the development process and close to final posting. Chemical assessments in Phase 2 of the implementation will address all of the short-term recommendations from Table F-1. The IRIS Program is

[11]Pub. L. No. 112-74, Consolidated Appropriations Act, 2012.
[12]NRC (2011) Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde.

implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Chemical assessments in Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined below in Table F-2, including the development of a standardized approach to describe the strength of the evidence for noncancer effects. On May 16, 2012, EPA announced[13] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

Table F-1. The EPA's implementation of the National Research Council's recommendations in the biphenyl assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the biphenyl assessment
General recommendations for completing the IRIS formaldehyde assessment that EPA will adopt for all IRIS assessments (see p. 152)	
1. To enhance the clarity of the document, the draft IRIS assessment needs rigorous editing to reduce the volume of text substantially and address redundancies and inconsistencies. Long descriptions of particular studies should be replaced with informative evidence tables. When study details are appropriate, they could be provided in appendices.	**Partially implemented.** Biphenyl is a post-peer review Phase 1 chemical; as such, implementation has focused on a subset of the short-term recommendations, such as editing and streamlining, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data. As a Phase 1 chemical, study summaries were not replaced by evidence tables; however, study summaries were edited to make them more concise and transparent. Summaries of genotoxicity studies and other mechanistic studies are provided in an appendix (Appendix C), with brief synthesis text on mode of action in the body of the Toxicological Review. Technical and scientific edits were performed to eliminate any redundancies or inconsistencies.
2. Chapter 1 needs to be expanded to describe more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria articulated and a better description of the outcomes of the searches and clear descriptions of the weight-of-evidence approaches used for the various noncancer outcomes. The committee emphasizes that it is not recommending the addition of long descriptions of EPA	**Partially implemented.** A section entitled Literature Search Strategy and Study Selection is provided as Appendix B. This appendix describes the search strategy used to identify the pertinent health effects literature and identifies EPA guidance used to guide selection of studies for hazard identification. Statements of criteria used to exclude, include, and advance studies for derivation of toxicity values are being developed as part of Phase 2.

[13]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris).

Table F-1. The EPA's implementation of the National Research Council's recommendations in the biphenyl assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the biphenyl assessment
guidelines to the introduction, but rather clear concise statements of criteria used to exclude, include, and advance studies for derivation of the RfCs and unit risk estimates.	
3. Standardized evidence tables for all health outcomes need to be developed. If there were appropriates tables, long text descriptions of studies could be moved to an appendix or deleted.	**Not implemented.** The assessment was largely finalized before the release of the NRC recommendations; thus, the tables herein are not consistent with standardized evidence tables. However, the main text of the assessment contains summary tables of the major toxicity studies of biphenyl (Tables 4-14 and 4-15).
4. All critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated and based on the type of research, for example, observational epidemiologic or animal bioassays. The findings of the reviews might be presented in tables to ensure transparency.	**Partially implemented.** All critical studies of biphenyl discussed in Chapter 4 and Appendix C were thoroughly evaluated. EPA guidance documents that were used to guide the evaluation of human and animal studies are identified in Sections 5.1.1 and 5.2.1 and in the appendix on Literature Search Strategy and Study Selection. Critical evaluation of the available experimental animal studies of biphenyl is included in Sections 4.6 and 4.7.1 (weight-of-evidence evaluations for noncancer and cancer effects, respectively), and Sections 5.1.1 and 5.2.1 as part of the consideration of studies selected for dose-response analysis. Standardized approaches for evaluating studies are under development as part of Phases 2 and 3.
5. The rationales for the selection of the studies that are advanced for consideration in calculating the RfCs and unit risks need to be expanded. All candidate RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.	**Partially implemented.** The text has been revised to provide expanded rationales for selection of the studies advanced for consideration in calculating the RfD and quantitative cancer risk estimate. The selection considerations for the RfD are also summarized in Table 5-4 and graphically in Figure 5-1. Because the available data were not adequate to support the derivation of an RfC, no studies were advanced for RfC derivation. More extensive use of graphical displays will be included in those assessments that are part of Phase 2.
6. Strengthened, more integrative and more transparent discussions of weight-of-evidence are needed. The discussions would benefit from more rigorous and systematic coverage of the various determinants of weight-of-evidence, such as consistency.	**Partially implemented.** Sections 4.6 and 4.7.1 have been substantially enhanced to provide more integrative and transparent discussions of the weight of evidence for noncancer and cancer effects of biphenyl, taking into consideration determinants such as consistency of effect across studies, sexes, and species, number of studies demonstrating a response, demonstration of dose-response, and study quality. A more rigorous and formalized approach for characterizing the weight of evidence will be developed as part of Phase 3.

Table F-1. The EPA's implementation of the National Research Council's recommendations in the biphenyl assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the biphenyl assessment
General Guidance for the Overall Process (see p. 164)	
7. Elaborate an overall, documented, and quality-controlled process for IRIS assessments.	**Partially implemented.** A team approach was used for the development of the biphenyl assessment to help ensure that the necessary disciplinary expertise was available for assessment development and review, and to provide a forum for identifying and addressing key issues. Because biphenyl is a post-peer review, Phase 1 chemical, the biphenyl team did not have access to the "overall, documented, and quality-controlled process" that is now being developed in response to the NRC recommendations.
8. Ensure standardization of review and evaluation approaches among contributors and teams of contributors; for example, include standard approaches for reviews of various types of studies to ensure uniformity.	
9. Assess disciplinary structure of teams needed to conduct the assessments.	
Evidence Identification: Literature Collection and Collation Phase (see p. 164)	
10. Select outcomes on the basis of available evidence and understanding of mode of action.	**Partially implemented.** The hazards associated with biphenyl exposure by the oral and inhalation pathways were selected based on identification and synthesis of the available evidence (see Section 4.6 for noncancer effects and Section 4.7 for cancer). A detailed discussion of mode of action for biphenyl-induced tumors is provided in Section 4.7.3. This mode-of-action analysis supports a nonlinear extrapolation approach for bladder tumors. The cancer outcome selected for quantitative analysis (mouse liver tumors) is consistent with the available evidence and mode-of-action findings.
11. Establish standard protocols for evidence identification.	**Partially implemented.** This is being implemented by the IRIS Program as part of Phase 2. The EPA's literature search strategy for evidence identification for biphenyl is provided in Appendix B, Literature Search Strategy and Study Selection, and Figure B-1.
12. Develop a template for description of the search approach.	**Not implemented.** This is being implemented by the IRIS Program as part of Phase 2.
13. Use a database, such as the Health and Environmental Research Online (HERO) database, to capture study information and relevant quantitative data.	**Implemented.** HERO links were incorporated for all citations.
Evidence Evaluation: Hazard Identification and Dose-Response Modeling (see p. 165)	
14. Standardize the presentation of reviewed studies in tabular or graphic form to capture the key dimensions of study characteristics, weight-of-evidence, and utility as a basis for deriving reference values and unit risks.	**Partially implemented.** This assessment was largely finalized before the release of the NRC recommendations; thus, the tables herein may not be consistent with current standardizations. The biphenyl assessment provides summary tables of the major toxicity studies of biphenyl (Tables 4-14 and 4-15) that includes

Table F-1. The EPA's implementation of the National Research Council's recommendations in the biphenyl assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the biphenyl assessment
	information on study protocols and results.
15. Develop templates for evidence tables, forest plots, or other displays.	**Not implemented.** This is being implemented by the IRIS Program as part of Phase 2.
16. Establish protocols for review of major types of studies, such as epidemiologic and bioassay.	**Partially implemented.** This is being implemented by the IRIS Program as part of Phase 2. The study review process was not revised for this assessment because biphenyl is a Phase 1 chemical. However, this assessment was developed using standard protocols for evidence evaluation that are provided in existing EPA guidance.
Selection of Studies for Derivation of Reference Values and Unit Risks (see p. 165)	
17. Establish clear guidelines for study selection. a. Balance strengths and weaknesses. b. Weigh human vs. experimental evidence. c. Determine whether combining estimates among studies is warranted.	**Partially implemented.** As discussed above, the text has been expanded to include more description of the considerations made in selecting the study that formed the basis for the RfD and oral slope factor (see Sections 5.1 and 5.4.1). Citations to EPA guidance documents that were used to guide study selection, including consideration of the strengths and weaknesses of individual studies, were provided. The biphenyl database did not support the combination of estimates across studies.
Calculation of Reference Values and Unit Risks (see pp. 165-166)	
18. Describe and justify assumptions and models used. This step includes review of dosimetry models and the implications of the models for uncertainty factors; determination of appropriate points of departure (such as benchmark dose, no-observed-adverse-effect level, and lowest observed-adverse-effect level), and assessment of the analyses that underlie the points of departure.	**Implemented as applicable.** The biphenyl assessment provides a detailed discussion of the dose-response modeling used to derive candidate points of departure for the RfD (see Section 5.1.2) and justification of the uncertainty factors applied to the point of departure (see Section 5.1.3). The assessment also provides a detailed discussion of model selection for liver tumor data used to derive the cancer slope factor (see Section 5.4.3). Dosimetry models are not available for biphenyl; therefore, a default method for extrapolating dose from animals to humans based on body weight to the ¾ power was used consistent with EPA guidance.
19. Provide explanation of the risk-estimation modeling processes (for example, a statistical or biologic model fit to the data) that are used to develop a unit risk estimate.	**Implemented as applicable.** A detailed discussion of model selection, evaluation of statistical model fit to the data, and cross-species scaling used to derive the oral cancer slope factor is provided in Section 5.4.3. An inhalation cancer unit risk was not derived.
20. Provide adequate documentation for conclusions and estimation of reference values and unit risks. As noted by the committee throughout the present report,	**Implemented.** The biphenyl assessment provides thorough documentation of the basis for the derivation of the RfD and cancer slope factor in Appendix D and E, respectively.

Table F-1. The EPA's implementation of the National Research Council's recommendations in the biphenyl assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the biphenyl assessment
sufficient support for conclusions in the formaldehyde draft IRIS assessment is often lacking. Given that the development of specific IRIS assessments and their conclusions are of interest to many stakeholders, it is important that they provide sufficient references and supporting documentation for their conclusions. Detailed appendixes, which might be made available only electronically, should be provided when appropriate.	

Table F-2. National Research Council recommendations that the EPA is generally implementing in the long term

NRC recommendations that EPA is implementing in the long-term	Implementation in the biphenyl assessment
Weight-of-Evidence Evaluation: Synthesis of Evidence for Hazard Identification (see p. 165) 1. Review use of existing weight-of-evidence guidelines. 2. Standardize approach to using weight-of-evidence guidelines. 3. Conduct agency workshops on approaches to implementing weight-of-evidence guidelines. 4. Develop uniform language to describe strength of evidence on noncancer effects. 5. Expand and harmonize the approach for characterizing uncertainty and variability. 6. To the extent possible, unify consideration of outcomes around common modes of action rather than considering multiple outcomes separately.	**Not implemented.** As indicated above, Phase 3 of EPA's implementation plan will incorporate the longer-term recommendations made by the NRC. On May 16, 2012, EPA announced that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. In addition, EPA will hold a workshop on August 26, 2013, on issues related to weight-of-evidence to inform future assessments.
Calculation of Reference Values and Unit Risks (see pp. 165-166) 7. Assess the sensitivity of derived estimates to model assumptions and end points selected. This step should include appropriate tabular and graphic displays to illustrate the range of the estimates and the effect of uncertainty factors on the	**Partially implemented.** As indicated above, Phase 3 of EPA's implementation plan will incorporate the longer-term recommendations made by the NRC, including assessment of the sensitivity of derived estimates to model assumptions and endpoint selection. In the biphenyl assessment, Section 5.1 presents alternative endpoints that were considered as candidates for the development of the RfD. Candidate points of departure

Table F-2. National Research Council recommendations that the EPA is generally implementing in the long term

NRC recommendations that EPA is implementing in the long-term	Implementation in the biphenyl assessment
estimates.	derived for these endpoints are presented in tabular form in Table 5-4 and in graphical form in Figure 5-1.