

PEER REVIEWER COMMENTS

**External Peer Review on the
Toxicological Review of Biphenyl
(CASRN 92-52-4)**

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I. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the draft “Toxicological Review of Biphenyl” that will appear on the Agency’s online database, the Integrated Risk Information System (IRIS). IRIS is an EPA database, prepared and maintained by EPA’s National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD), which contains potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances.

The existing IRIS assessment for biphenyl includes an RfD posted in 1989 and a cancer weight-of-evidence descriptor posted in 1991. However, the existing IRIS assessment has been updated and includes an RfD and a cancer assessment. The external review draft “Toxicological Review of Biphenyl” has undergone EPA review for scientific accuracy and compliance with EPA risk assessment guidelines and procedures; the next step in the process is an external peer review.

PEER REVIEWERS

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Dr. Bartell is currently Assistant Professor in the Program of Public Health, Department of Statistics, and Department of Epidemiology at University of California, Irvine. Dr. Bartell received his Ph.D. in Epidemiology from the University of California, Davis in 2003 and holds Master’s degrees in both Statistics (2001) and Environmental Health (1996). Prior to joining the faculty at UC Irvine in 2006, Dr. Bartell served as an Assistant Professor at Emory University (2003-2006) where he continues to serve as an Adjunct Assistant Professor. Dr. Bartell’s research interest is environmental health methodology including statistical methods for exposure assessment, environmental epidemiology, and risk/decision analysis. Dr. Bartell has served on a variety of scientific advisory committees for the National Research Council, the Environmental Protection Agency, the Centers for Disease Control and Prevention, the National Institute of Environmental Health Sciences, and the Department of Energy. He has been a manuscript referee for over 10 journals, including American Journal of Epidemiology, Cancer Epidemiology, Environmental Health Perspectives, and Statistics in Medicine.

John M. Cullen, Ph.D., V.M.D.

Dr. Cullen is Alumni Distinguished Undergraduate Professor in the Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, where he has been a member of the faculty since 1984. Dr. Cullen received his Ph.D. in Comparative Pathology from University of California, Davis in 1985, before that having received a V.M.D. from the University of Pennsylvania in 1975. From 1979-1984, he was resident, and then senior resident of Anatomic Pathology at University of California, Davis’ Veterinary Medical Teaching Hospital. Dr. Cullen is a board certified Diplomate of the American College of Veterinary Pathology (1982) and holds a veterinary license in the state of North Carolina. His current research is in hepatic toxicity (comparisons of acute hepatotoxicity in conventional and germ free mice, acute biliary toxicity) and animal models of viral hepatitis.

Dr. Cullen has published numerous papers, book chapters, and proceedings and has been an invited presenter for many organizations around the world. Recent presentations include *Hepatic Pathology of Small Animals* (Ecole Veterinaire, Maisons d'Alfort, Paris, France, June 2011) and the keynote presentation, *Time Course Analysis of Laser Capture Microdissection and Gene Expression in Acute ANIT Toxicity*, at the Annual Meeting of the European Society of Toxicologic Pathology and the European Society of Veterinary Pathology, Uppsala, Sweden, 2011. He reviews articles for publications such as *Cancer Research*, *Contemporary Topics in Laboratory Animal Medicine*, *Journal of Veterinary Pharmacology*, and *Toxicologic Pathology*, and was a member of the editorial board for *Veterinary Pathology* from 1996-2000. Dr. Cullen's professional memberships include the American College of Veterinary Pathologists, Society of Toxicologic Pathologists, American Association for the Study of Liver Disease, and American Veterinary Medical Association.

Brant A. Inman, M.D., M.Sc., FRCS(C)

Dr. Inman is Assistant Professor of Urology at Duke University Medical Center and Durham VA Medical Center, in Durham, North Carolina. He received his Doctor of Medicine from University of Alberta, Canada in 2000. He completed an Anatomic Pathology Internship at the University of Calgary, Calgary, Alberta, Canada in 2001 and subsequently completed his residency in Urology at the Université Laval, Québec, Québec, Canada in 2005. Dr. Inman has also held a Urologic Oncology Fellowship at Mayo Clinic College of Medicine, Rochester, Minnesota (2005-2008) and earned a Master of Clinical and Translational Science from the Mayo Graduate School (2011). Dr. Inman's research focuses on urothelial tumors, novel diagnostics and therapeutics, as well as prostate cancer management. He has published numerous peer-reviewed articles, editorials and book chapters. He currently serves as interim Vice Chief of Research for the Division of Urology. Among others, Dr. Inman's professional memberships include Alberta Medical Association, American Association for Cancer Research, American Society for Clinical Oncology, American Urological Association, and the Society for Urologic Oncology. He is on the Editorial Board for *European Urology* and serves as a reviewer for publications such as *British Journal of Urology*, *Journal of American College of Surgeons*, *Journal of Urology*, *Prostate Cancer and Prostatic Diseases*, and *Urologic Oncology*.

Frederick J. Miller, Ph.D., Fellow ATS

Dr. Miller is Adjunct Medical Research Professor at Duke University Medical Center, Durham, North Carolina, in addition to owning and operating his own consulting company, Fred J. Miller & Associates, LLC. He previously served as the Vice President for Research at CIIT Centers for Health Research, Research Triangle Park, North Carolina (2002-2005) and Medical Research Professor at Duke (1990-2008). He was a member of CIIT Centers for Health Research board of directors from 2003 to 2005. He was a U. S. Public Health Service officer assigned to the EPA's Health Effects Research Laboratory, Research Triangle Park, North Carolina, from 1973 to 1989. While with EPA, he held various management and supervisory positions, including Director of the Inhalation Toxicology Division. Dr. Miller received his Ph.D. in Statistics from North Carolina State University (1977) after having earned a Master's in Statistics from the University of Wyoming in 1968. He has 165 publications and book chapters and has served on many advisory and consultation panels for EPA, as well as other organizations. He has participated in IRIS reviews both as an external reviewer and as an SAB member. In 2005, Dr.

Miller received the Career Achievement Award from the Inhalation Specialty Section of the Society of Toxicology. He is a Fellow of the Academy of Toxicological Sciences and serves on the Editorial Board of Inhalation Toxicology.

Ricardo Saban, D.V.M., Ph.D.

Dr. Saban is currently a Professor in the Department of Physiology and an Adjunct Professor of Obstetrics and Gynecology in the College of Medicine at the University of Oklahoma Health Sciences Center. He is also a Visiting Professor in the Departments of Surgical Sciences and Neurosciences at the University of Wisconsin-Madison. Prior to joining the faculty at the University of Oklahoma, Dr. Saban was associated with the University of Texas Medical Branch and the University of Wisconsin-Madison. Dr. Saban received a Livre Docteur in Pathology in 1986, a Ph.D. in Physiology in 1979, and a D.V.M. in 1972 from the University of São Paulo in Brazil. He completed Postdoctoral Fellowships at the University of Wisconsin-Madison in Pharmacology (1982-1984) and in Physiology (1986-88). Dr. Saban's current research interest is on inflammation and cancer-induced lymphangiogenesis. His research has focused on identifying organ and disease specific genes, transcription factors, and regulatory networks using various tools such as disease animal models, tissue specific promoters, molecular imaging, suppression subtractive hybridization, cDNA arrays, and transcriptional factors analyses. Dr. Saban has been an invited speaker at over 30 symposiums/meetings and has published over 100 journal articles and numerous book chapters. He is on the Editorial Board of The American Journal of Physiology-Renal and serves as an editor on a dozen other scientific journals. Dr. Saban's professional memberships include the American Urologic Association, the American Physiological Society, and the Society of Basic Urologic Research.

Mary Alice Smith, Ph.D.

Dr. Smith is an Associate Professor in the Department of Environmental Health Science at the University of Georgia where she also serves as Core Faculty at the Regenerative Biosciences Center and the Center for Food Safety. Dr. Smith received her Ph.D. in Toxicology in 1990 from the University of Arkansas for Medical Sciences and received an M.S. in Biology (concentration in Developmental Biology) from Emory University. Her current research focuses on the effects of agents on pregnancy and development, developing in vitro and in vivo models for developmental toxicity testing and using that data in assessing risk. Her research incorporates use of animal models and in vitro models for pregnancy and development, determination of dose response for adverse effects, use of embryonic stem cells and metabolomics to predict adverse effects, and use of data to develop risk assessments for microbial and chemical exposures during pregnancy. She teaches undergraduate and graduate courses in toxicology, developmental toxicology and risk assessment. Dr. Smith has authored over 50 papers and book chapters and has been an invited speaker at over 50 international, national, and state/regional meetings. Dr. Smith has served on review panels for the National Academy of Sciences, the National Institutes of Health, the Agency for Toxic Substances and Disease Registry and the Environmental Protection Agency. She is a reviewer for over ten professional journals including Reproductive Toxicology, Risk Analysis and the Journal of Food Protection. Dr. Smith is currently a member of the Teratology Society, Society of Toxicology, American Society of Microbiology, and the International Association for Food Protection.

Paul W. Snyder, D.V.M., Ph.D., DACVP

Dr. Snyder is a Professor of Veterinary Pathology in the Department of Comparative Pathobiology, School of Veterinary Medicine, at Purdue University and an Adjunct Professor of Pathology and Laboratory Medicine at the Lafayette Center for Medical Education in the Indiana University School of Medicine. He also serves as Director of the Purdue Histology and Phenotyping Laboratory. Dr. Snyder received his D.V.M. from Iowa State University in 1985 and completed his Veterinary Pathology Residency at the University of Illinois in 1989. Dr. Snyder received his Ph.D. in 1992 at Purdue University and is certified as a Diplomate of the American College of Veterinary Pathologists. He is also a licensed veterinarian in three states (Iowa, Indiana, and Wisconsin). His current research in veterinary pathology includes immunopathology, immunotoxicology, toxicologic pathology, developmental biology, and environmental medicine. Dr. Snyder has published over 150 journal articles and reports, as well as book chapters. He has been an invited speaker at numerous international and national meetings and symposiums. He is also a Fellow of the International Academy of Toxicology Pathology. Dr. Snyder's professional memberships include the American College of Veterinary Pathologists, Society of Toxicologic Pathologists, and the American Veterinary Medical Association.

Lauren Zeise, Ph.D.

Dr. Zeise has been Chief of the Reproductive and Cancer Hazard Assessment Branch of the California Office of the Environmental Health Hazard Assessment (OEHHA) since 1991. She received her Ph.D. in 1984 from Harvard University. She oversees a variety of California's risk assessment and public health activities, including cancer and reproductive toxicant assessments; development of frameworks and methodologies for assessing cumulative impacts, nanotechnology, green chemistry/safer alternatives, and susceptible populations; the California Environmental Contaminant Biomonitoring Program; and health risk characterizations for environmental media, food, fuels and consumer products. Dr. Zeise's research focuses on human interindividual variability and risk. Dr. Zeise has been an invited speaker at over 100 meetings and workshops and has authored over 100 journal papers and books/chapters. She was the 2008 recipient of the Society of Risk Analysis Outstanding Practitioners Award and is a National Associate of the National Academy of Science's National Research Council. She has served on various advisory boards and committees of the US EPA, Office of Technology Assessment, World Health Organization, and National Institute of Environmental Health Sciences. She has also served on a number of NRC and IOM committees and boards, including the committee that produced *Toxicity Testing in the 21st Century: A Vision and Strategy*, *Science and Decisions: Advancing Risk Assessment*, and *Understanding Risk: Informing Decisions in a Democratic Society*.

II. CHARGE TO REVIEWERS

EPA is seeking an external peer review of the draft “Toxicological Review of Biphenyl” that will appear on the Agency’s online database, IRIS. IRIS is prepared and maintained by EPA’s National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). The existing IRIS assessment for biphenyl includes a chronic reference dose (RfD) posted in 1989 and a cancer weight-of-evidence descriptor posted in 1991. The external review draft “Toxicological Review of Biphenyl” includes an RfD and a cancer assessment.

Charge Questions

Below is a set of charge questions that address scientific issues in the draft “Toxicological Review of Biphenyl.” Please provide detailed explanations for responses to the charge questions. EPA will also consider reviewer comments on other major scientific issues specific to the hazard identification and dose-response assessment of biphenyl. Please identify and provide the rationale for approaches to resolve the issues where possible. Please consider the accuracy, objectivity, and transparency of EPA’s analyses and conclusions in your review.

General Charge Questions:

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

Chemical-Specific Charge Questions:

(A) Oral reference dose (RfD) for biphenyl

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.
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, 2000)? 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?

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.

(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.

(C) Carcinogenicity of biphenyl

1. Under EPA’s *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005; www.epa.gov/iris/backgrd.html), 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.
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.
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).

Oral Slope Factor (OSF)

4. A two-year cancer bioassay of biphenyl in BDF₁ 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.

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.
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, 2000)? 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?
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.

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.

III. GENERAL IMPRESSIONS

Scott M. Bartell

This toxicological review is generally well written and quite readable (more so than other recent toxicological reviews). However, I do have some suggestions as noted below. Among other issues the tables of raw data from previous studies are rife with statistical errors and oddities (see specific comments below). These don't appear to affect the substantive conclusions of the report, but they should be fixed nonetheless.

I am not a toxicologist or a subject expert for biphenyl, so I cannot evaluate the accuracy of the literature review or scientific support on these topics. The following comments are largely focused on clarity of the overall document and substantive issues in dose-response modeling, uncertainty analysis, and statistics.

John M. Cullen

The Toxicological Review is well written and clearly presents the relevant data. I was not able to find any relevant literature that was not included in the review. Overall, the report was well done. There was a clear effort to identify appropriate documents that included recent and some studies that were quite old. The writing style was straightforward and easy to follow. The incorporation of various data tables was an effective presentation technique. The appendices provided a thorough compilation of background data as well. I could follow the process that led to the conclusions presented in the document, but have some residual questions concerning the relative weight that was placed of different studies. More detail on the strengths and weakness of some of the older studies compared to the recent ones as well as the process by which they were rated would be valuable. Some perspective on actual human exposure compared to levels of exposure in experimental animals would be useful to aid in the assessment of actual and reasonable human risks.

Brant A. Inman

The document is an expansive and thorough review of the available data concerning the health risks of biphenyl. While the data presented was almost exclusively from non-human studies, the small amount of human data that was summarized seemed compelling enough to me to justify the thorough review of the animal data.

While on the whole I found the document organized and structured, I also found it repetitive and often redundant. I was frequently reading the same data presented in different ways and this on more than one occasion caused confusion as I was unsure whether I was reading new data or previously presented data that was just being rehashed under a different subheading of the document. I would have preferred a less repetitive (and probably much shorter) manuscript. Other minor annoyances were the usage of outdated terminology throughout the manuscript (more on this problem below) and the frequent usage of abbreviations. I personally prefer that abbreviations be minimized in all scientific documents because their prevalence can make a document unreadable except to the authors that wrote it.

More importantly, however, the data presented in the document convinced me that biphenyl is a serious potential health concern for humans with numerous potential adverse effects. The more concerning effects for me were the urinary toxicities, the hepatic toxicities, and the risks to developing fetuses. These are real concerns for humans.

Frederick J. Miller

Overall, the biphenyl toxicity document is logically organized, and the material within the various sections is clearly presented. The conclusions reached by the authors are scientifically defensible relative to the selection of the critical studies from both the oral and inhalation routes. However, the Agency has not adequately defended their choice of using liver tumors in female mice to derive the oral slope factor; there is no discussion of the major discrepancy in the selected study between the diametrically opposite results seen in male mice versus female mice. The decision by the Agency to not develop a RfC is an appropriate one given the lack of data that would need to be available to do so. The BMD appendix provides supportive data for the discussion of the modeling results that are presented in the main body of the report.

As with various other IRIS documents, this document suffers from a lack of the Agency providing any description of environmental biphenyl exposure levels. The Agency should add material on exposure levels so readers gain a better perspective on what margin there is between any derived RfD, RfC, and IUR values and actual human biphenyl exposures from the oral and inhalation routes. Statements are made in connection with categorizing biphenyl as “suggestive evidence of carcinogenic potential” at environmentally relevant exposure levels that are: (1) not supported by exposure data, (2) factually incorrect, and (3) highly misleading. The reader would be able to see this easily if the document contained exposure data. For example, Canadian exposure data given in Table 1 below illustrate this and were obtained at <http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/biphenyl-biphenyle/evaluation-eng.php>.

Table 1: Upper-bounding estimates of daily intake of biphenyl by the general population of Canada

Route of exposure	Estimated intake ($\mu\text{g}/\text{kg}\text{-bw}$ per day) of biphenyl by various age groups						
	0-6 months ^{1, 2, 3}		0.5-4 years ⁴	5-11 years ⁵	12-19 years ⁶	20-59 years ⁷	60+ years ⁸
	formula fed	not formula fed					
Ambient air ⁹	7.7×10^{-4}		1.7×10^{-3}	1.3×10^{-3}	7.3×10^{-4}	6.3×10^{-4}	5.5×10^{-4}
Indoor air ¹⁰	0.25		0.53	0.41	0.23	0.2	0.17
Drinking water ¹¹	3.4×10^{-3}	1.3×10^{-3}	1.4×10^{-3}	1.1×10^{-3}	6.4×10^{-4}	6.7×10^{-4}	7.1×10^{-4}
Food and beverages ¹²	0.98		0.97	0.74	0.43	0.33	0.28
Soil ¹³	3.2×10^{-5}		5.2×10^{-5}	1.7×10^{-5}	4.0×10^{-6}	3.4×10^{-6}	3.3×10^{-6}
Total intake	0.25	1.22	1.5	1.15	0.67	0.53	0.46

1. No data were identified on concentrations of biphenyl in breast milk.
2. Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (EHD, 1998).
3. For exclusively formula-fed infants, intake from water is synonymous with intake from food. The concentration of biphenyl in water used to reconstitute formula was based on Williams et al. (1982). No data on concentrations of biphenyl in formula were identified for Canada. Approximately 50% of non-formula-fed infants are introduced to solid foods by 4 months of age and 90% by 6 months of age (NHW, 1990).
4. Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (EHD, 1998).
5. Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (EHD, 1998).

6. Assumed to weigh 59.4 kg, to breathe 15.8 m³ of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (EHD, 1998).
7. Assumed to weigh 70.9 kg, to breathe 16.2 m³ of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (EHD, 1998).
8. Assumed to weigh 72.0 kg, to breathe 14.3 m³ of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (EHD, 1998).
9. The highest concentration of biphenyl measured in outdoor air along the Niagara River in Fort Erie, Niagara Falls and Niagara-on-the-Lake, Ontario, was 0.022 µg/m³ (Hoff and Chan, 1987). Canadians are assumed to spend 3 hours outdoors each day (EHD, 1998). This concentration is within the range of concentrations reported in another study of outdoor air in Canada (Patton et al., 1991) and many studies in the United States and Norway.
10. The concentration of biphenyl in indoor air, based on a composite of 757 indoor air sample extracts taken from Canadian residential homes, was 1 µg/m³ (Otson et al., 1994). Canadians are assumed to spend 21 hours indoors each day (EHD, 1998). This concentration is within the range of concentrations reported in studies of indoor air in Canada (Otson and Benoit, 1986), the United States (Wilson et al., 2001) and Finland (Kostiainen, 1995).
11. The highest concentration of biphenyl measured in 24 samples of drinking water from 12 Great Lakes municipalities in Ontario was 0.0319 µg/L (Williams et al., 1982). This was the highest value reported in the available studies carried out in Canada (Benoit et al., 1979a, 1979b; LeBel et al., 1987; City of Toronto Water and Wastewater Services Division, 2002a, 2002b, 2002c, 2002d).
12. Estimates of intake from food are based upon concentrations in foods that are selected to represent the 12 food groups addressed in calculating intake (EHD, 1998): Dairy products: 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Fats: 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Fruits and fruit products: 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Vegetables: 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Cereal products: 48 µg/kg; maximum concentration of five samples of oats, whole grain imported from Canada (U.S. FDA, 2003b) Meat and poultry: 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Fish: 2.64 µg/kg; maximum concentration in 239 samples of freshwater fish from the Northwest Territories (Braune et al., 1999) Eggs: 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Foods, primarily sugar: 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Mixed dishes: no data identified Nuts and seeds: 7 µg/kg, highest concentration of biphenyl in 53 samples of cashews in the U.S. survey (U.S. FDA, 2002b) Beverages (soft drinks/alcohol/coffee/tea): 5 µg/kg; detection limit in U.S. survey (U.S. FDA, 2002a, 2003a) Amounts of foods consumed on a daily basis by each age group are described by Health Canada (EHD, 1998).
13. No data for biphenyl in soil in Canada were identified. The highest concentration of biphenyl found in dust samples collected at day care centres in Raleigh-Durham-Chapel Hill, North Carolina, was 8 µg/kg (Wilson et al., 2001). Although higher concentrations were reported in Norway (Vogt et al., 1987; Aamot et al., 1996), the data were not as recent, and U.S. data are considered more appropriate for estimating levels in Canadian soil.

To this reviewer, the lack of environmental exposure data is a major deficiency. Since the Agency is reconsidering the content and form of IRIS documents, including a chapter or section on what is known about environmental exposures to the compound being reviewed should become part of the new template for these documents. Readers need to be able to understand the margin of exposure difference between the RfD and RfC and environmental exposures whether current or past.

Ricardo Saban

The information presented was a result of a detailed analysis of the available scientific information on biphenyl. However, the document is repetitive and a chapter synthesizing the information along with the sound conclusions would have improved its understanding. Overall,

this document was not clearly edited for reducing the repeated information. As an example: Page 81, lines 27-29 are unnecessary repetition of page 80, lines 10-12.

Mary Alice Smith

Overall the “Toxicological Review of Biphenyl” is well written and information is presented in tables and figures that are clear. The studies are described in detail, including some studies that probably would not have to be included. Some redundancy could be eliminated to make the report more concise and easier to read. The main concern is regarding the calculations of the RfD from data based on cumulative skeletal defects, some of which are being questioned by developmental toxicologists as ‘adverse’ effects. This was not discussed in the text and probably should have been, given the importance of this study to the overall conclusion of the document. More details regarding these concerns are given below. However, descriptions of mechanisms of action are very helpful.

Paul W. Snyder

The preliminary draft “Toxicological Review of Biphenyl” (CAS No. 92-52-4) presents a body of toxicological relevant data in an organized and logical manner. The information with this document is largely relevant and presented in a concise and accurate manner. Data are a compilation of the scientific literature from peer reviewed published studies and scientific technical reports from a literature search strategy based on the chemical name, CAS registry number, and common synonyms. Additional scientifically relevant information, provided by the public, was also included. Those data cited within the draft report are primarily presented in summary form or abbreviated study descriptions, and in no instance provide raw data such as study protocols or results. As indicated in this draft report in some instances, published studies reported findings, but data in support of the findings were not included. The report summarizes the significant findings from animal and non-animal studies, and proposed mechanisms, known and unknown, for the findings. Relevance of the findings and proposed mechanisms to humans is discussed. This information is then utilized as evidence for dose-response assessments and human hazard potential for non-cancer and cancer endpoints.

Lauren Zeise

The Toxicological Review has done an excellent job assembling and synthesizing the available studies on biphenyl toxicity. The descriptions of the studies are at the right level, and directed at the critical issues for hazard identification and dose response. The various lines of evidence on mode of action has been thoughtfully considered and weighed. The writing is very good throughout. New information provides for the updating of the RfD, changing of the cancer hazard call, and derivation of an oral slope for the biphenyl. But there is much that is not known about the mode of action, pharmacokinetics, inhalation toxicity and possible genotoxicity of the compound. While most of the conclusions seem adequately supported, some deserve reconsideration or could be supported somewhat differently, given the uncertainties, as indicated in responses below.

IV. RESPONSE TO CHARGE QUESTIONS

General Charge Questions

Question 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?

Scott M. Bartell

Apart from statistical errors and some difficulties in the weight-of-evidence classification as noted below, the toxicological review is logical, clear, and concise; the scientific evidence is generally well synthesized.

John M. Cullen

The Toxicological Review is well written and clearly presents the relevant data. I was not able to find any relevant literature that was not included in the review. Overall, the report was well done. The integration of the more recent studies with the conclusions and their application to the various analyses was well done. There remains some question of how to evaluate the older studies on cancer and non-cancer endpoints as there could be more detail on the strengths and weaknesses of these studies in some organized stratification and some explanation as to their individual contributions to the final decision making. For example, there is one study showing liver tumor formation in biphenyl-treated female mice, but there are several studies in which no tumors were produced. Some greater explanation as to why this one study was regarded as the most reliable of the group would be important. Older studies often were performed without careful evaluation of the health status of the laboratory animals. Unexpected and unexplained deaths, for example can interfere with the correct statistical interpretation of the chemical effects if appropriate corrections are not employed. These studies often contained gaps in the information as to the strain, gender or diet of study animals. Clearly, the older studies are different than contemporary studies, but some form of ranking would be quite helpful.

In addition, material on exposure levels should be included to provide a better perspective on what margin there is between any derived RfD, RfC, and IUR values and actual human biphenyl exposures from the oral and inhalation routes to aid in the assessment of risk.

In other aspects of the document, I found the data review, the conclusions, and the support for the conclusions provided in the appendices, thorough and well thought out.

Brant A. Inman

I felt that the document presented a logical and thorough review of the toxicological data concerning biphenyl. However, the document was expansive in my opinion, not concise. Additionally, due to the redundant presentation of data, I was frequently confused by the data and had to skip to previous sections to verify whether the various sections were presenting similar data or not. The same data reappeared in slightly different forms in several places.

Frederick J. Miller

The toxicological review is an adequate assessment of the noncancer and cancer health effects of biphenyl. The Table of Contents provides a clear guide to the various sections of the document. There is a logical development of the document relative to the order in which the information is presented and there is adequate cross-referencing to earlier sections when the authors get to the dose-response assessments in Section 5.

Ricardo Saban

The Toxicological Review is well written, but it is not concise. Each chapter is a long description of findings regarding biphenyl. In some instances, the document is repetitive which clouds the central idea. It would be of a great contribution if at the end of each chapter a clear and concise conclusion had been made.

Mary Alice Smith

The Toxicological Review is clear and logically presented. A description of the search strategy for locating relevant literature, and how the searches were done, would lend more confidence that the searches were complete. There are some subheadings which could benefit from introductory paragraphs to indicate what studies will be discussed. One example is Section 4.2.1.2.3 Chronic studies in other animal species. The first sentence begins describing a study in dogs, whereas, it would help the reader to know what other species will be discussed and what endpoints will be evaluated. There are several sections which would benefit from this type of introductory paragraph. The overall synthesis of the information is adequate; however, some important considerations concerning endpoints (fetal skeletal defects and bladder cancers in rats) need to be discussed. There is also some redundancy in the text.

Paul W. Snyder

The Toxicological Review is logical, clear, and concise. The EPA has clearly presented and synthesized the scientific evidence for non-cancer and cancer health effects of biphenyl. Some minor modifications to the report could be made to clarify how the data should be interpreted in the context of human health risks.

Lauren Zeise

“3. TOXICOKINETICS” is well written, and addressed the key toxicokinetic considerations related to hazard identification and dose response. It would be helpful to have a closing subsection to section that summarized the key elements related to hazard and dose response. The section could be a short paragraph, at a very high level and, for example, could give conclusions regarding the extent of absorption, persistence in tissues, elimination pathways, CYPs apparently involved in metabolism, potential for reactive metabolite formation, and extent of formation. A key issue – initial distribution to target tissues – cannot be addressed because there are no data. (For example, toxicokinetic cannot help in considering neurotoxicity endpoints suggested in human studies.) Also, no toxicokinetic inhalation data are available. Some

comment about data limitations could also be made in the suggested closing piece on toxicokinetic section.

“4. HAZARD IDENTIFICATION” is very well written, clear and concise. Some suggestions for reconsideration of positions taken are provided in response to charge questions below. Suggestions for presentation or clarification are provided below in section V (Specific Observations). Some points beyond those provided in response to charge questions below are provided here:

- The incidence of reticular cell sarcoma in the biphenyl treated female Strain B mice shown in Table 4-9 is significantly greater than in controls by Fisher Exact Test ($p < 0.03$). This should be noted in Table 4-9 and discussed briefly in the text. It is interesting that for the case report of the worker dying from over exposure, the autopsy showed bone marrow damage. The occurrence of the tumor in mice deserves a mention in Section 4.7 EVALUATION OF CARCINOGENICITY.
- The non-rodent oral studies reported in section 4.2.1.2.3 are shorter than one-tenth the lifespan of the animal species and thus can be considered short term studies. Although 1 year dog studies can sometimes be referred to as chronic studies, U.S. EPA has noted that “The so-called chronic study in dogs is actually a short-term study, as it does not cover at least 10% of the life span” (A Review of the Reference Dose and Reference Concentration Processes, U.S. EPA, 2002, see pp. 3-9 and 3-10). A similar statement could be made about the 1 year study in rhesus monkeys (Dow et al., 1953). It would be preferable to include these studies in a different section than the “Chronic toxicity and carcinogenicity studies” section. In terms of lifespan they comprise less of the lifespan than is the case for a 90 rat study. Further, the group size in these studies is small: Male group sizes of two, female group size of one. One possibility would be to give them their own section (e.g., one year dog and rhesus monkey studies). Another possibility would be to include them in the subchronic study section.
- The overall weight of evidence for genotoxicity appears more equivocal than negative, given the clastogenicity findings in human lymphocytes, the in vivo findings, and the limited evidence for genotoxicity of metabolites.
- Regarding statements in the MOA section regarding lack of human and lab animal concordance for neurotoxicity, the animal studies were not designed to detect the neurotoxicity seen in human studies.
- The meaning of “environmentally relevant exposure levels in humans” (e.g., on pages 60 and 61) needs more description, and whether or not it is seen to apply to exposures in the occupational setting. Is “environmental” being used in the narrow sense of exposures of interest to U.S. EPA, or in the broader sense of all non-endogenous biphenyl exposures, including occupational?

General Charge Questions

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

Scott M. Bartell

I am not aware of any such studies.

John M. Cullen

I could find none. **skeletal changes papers

Brant A. Inman

I did not identify any additional studies.

Frederick J. Miller

A literature search of a number of databases revealed no new inhalation studies that could be brought to bear on the development of an RfC.

Ricardo Saban

I could not find any additional studies.

Mary Alice Smith

Although not directly related to biphenyl, an issue of Birth Defects Research was devoted to “Interpreting skeletal malformations and variations” (Birth Defects Research Part B Volume 80 (6), 2007. The articles in this volume directly address some of the malformations found in the Khera et al., 1979 study and should be considered. These articles directly impact whether the endpoint of skeletal malformations should be used for calculation of the RfD.

Paul W. Snyder

None.

Lauren Zeise

I did not identify any important studies on the health effects of biphenyl to include.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Biphenyl

Question 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.

Scott M. Bartell

The selection of the Khera et al. (1979) study is clearly described.

John M. Cullen

The study by Khera et al. (1979) appears to be the best available study on the developmental toxicity of biphenyl, although it is more than 30 years old. It would be helpful to have some discussion of the standards of the study design and any limitations to the quality of the data, as well as why the Agency went beyond the original conclusions of the study. Given that there is controversy regarding the validity of using the Wistar rat fetal bone development changes as adverse effects, it seems that the renal lesions (other than the hemosiderin deposition) would be a better reference point for this analysis (Umeda et al., 2002).

Brant A. Inman

This appeared to be an appropriate study for me and the justification provided for its use seems to make sense (it was the only oral developmental toxicity study found). However, at nearly 35 years old the study does seem dated and I wonder if more modern results would be similar.

Frederick J. Miller

The Agency selected the developmental study by Khera et al. (1979) as the basis for the derivation of the RfD because BMD modeling showed this study produced the lowest BMDL. The description of how the study was conducted was adequate to judge that the study was scientifically defensible. The gavage protocol allowed precise doses to be established for the various treatment groups, which was a strength of the study.

Ricardo Saban

The uncertainty factors appear to be reasonable. The selection of the study of Khera et al. indicates that no new study is available. This subject is outside my area of expertise.

Mary Alice Smith

As mentioned above, some of the skeletal effects are not considered adverse unless accompanied by other fetal abnormalities. Some discussion of this should be included. It would be helpful to

know what anomalies were included in categories such as ‘anomalous litters.’ Although this study may be the appropriate study for calculation of the RfD, it is somewhat difficult to determine without more details on these categories, which are not included in the original publication. The Umeda et al. 2002 study appears to be more scientifically justifiable for the RfD calculation.

Paul W. Snyder

The Khera et al. (1979) study was a rat gavage study that identified a NOAEL of 500 mg/kg-day for maternal toxicity and 250 mg/kg-day for developmental effects. The developmental effects were identified as unossified or delayed ossification findings. Such fetal skeletal effects are highly variable within a study and can be influenced by sampling procedure, maternal toxicity, and normal individual variation. Maternal toxicity is frequently manifested as decreased body weights and decreased body weight gains. There are numerous robust dietary studies that would be more appropriate and selecting the one with the lowest NOAEL would be appropriate (Umeda 2002).

Lauren Zeise

This study is a reasonable choice for RfD development. Another reasonable choice is the Umeda et al. (2002) study, with kidney hemosiderin deposit as the critical endpoint. The BMD is lower, and BMDL is a little higher, indicating a tighter confidence bound.

(A) Oral Reference Dose (RfD) for Biphenyl

Question 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.

Scott M. Bartell

The selection of fetal skeletal anomalies as the critical effect for derivation of the RfD is clearly described.

John M. Cullen

This is a difficult issue. I have reviewed one of the references provided by the ACC and written by Carney and Kimmel (2007). They argue that delayed ossification is not adverse as there is no long term developmental anomaly associated with late ossification and that this process can be driven by maternal stress which is essentially a non-specific effect. Given that there was death in the rats given the next highest dose, maternal stress seems likely. Maternal risk was only evaluated by body weight and mortality and more subtle, but significant clinically relevant indicators could have detected evidence of maternal stress. The process behind the choice to use litter-based skeletal abnormalities should be expanded and defended or the renal lesions could be substituted for this analysis.

Brant A. Inman

Since there is only 1 study of oral developmental toxicity (Khera et al., 1979) and the only non-lethal effect seen on the fetuses was skeletal anomalies (at doses ≥ 500 mg/kg/d), I think that the choice is appropriate.

Frederick J. Miller

The adversity of the fetal skeletal anomalies was discussed, and the authors acknowledged that there is some question as to the direct translatability of these rat findings to humans. Extensive discussions at the External Peer Review meeting on April 3, 2012 brought out that the Agency should not rely on fetal skeletal anomalies for the derivation of the RfD. Rather, there appears to be more scientifically defensible reasons for considering the kidney effects (i.e., simple hyperplasia, renal pelvis mineralization, papillary mineralization) seen in rats of both sexes for which the BMDL10 is quite consistent. The hemosiderin effect in female rats was judged by panel members to be a non-specific effect that usually is meaningless relative to humans.

Interactions of panel members with EPA staff in attendance at the meeting on April 3rd identified that the Agency was relying, in part, on the 1991 "Guidelines for Developmental Toxicity Risk Assessment" document. New scientific evidence and current interpretation of effects need to be incorporated in updated guideline documents that the Agency utilizes. For example, the paper

entitled “The relationship of maternal and fetal toxicity in developmental toxicology bioassays with notes on the biological significance of the “no observed adverse effect level” by Chernoff, N., Rogers, E. H., Gage, M.I., and Francis, B. M., that appeared in 2008 in *Reprod. Toxicol.* 25:192-202, has significant implications for developmental toxicity risk assessments.

Ricardo Saban

During the discussion of this specific question, several suggestions were raised and I agree with the discussants, particularly with the suggestion that “the discussion regarding the choice to use this effect could be expanded” as indicated by Dr. Cullen.

Mary Alice Smith

As described in Question 1 (A) above, more detailed description of what is included in ‘anomalous litters’ is needed. The highest dose had maternal toxicity and this needs to be considered in the RfD selection. At minimum, a thorough discussion of whether delayed ossification and extra ribs are considered an ‘adverse’ effect by developmental toxicologists should be included with the recognition that, in the future, these endpoints may not be considered adverse.

The Review reproduces Table 2 from the original publication (Khera et al., 1979) with slight modifications. However, Khera et al. (1979) states that the only indication of fetotoxic effects was at the highest dose group (1000 mg/kg) which produced maternal toxicity, and even so, the effects were not statistically significant at $p < 0.05$. The Review used a different statistical test (Fisher’s exact) and found statistical differences. There should be some discussion that the original paper found no statistical differences and justification for using the Fisher’s exact test. This is important, given that this endpoint was used to calculate the RfD.

Paul W. Snyder

Fetal skeletal anomalies, identified as delayed sternebrae ossification in this study, could be attributable to maternal toxicity as evidenced by the decreased body weights and decreased body weight gains in the 500 mg/kg/day group. The combination of a high background incidence and evidence of maternal toxicity make this study less robust for identifying a critical effect in deriving the RfD. The robust oral study with the lowest NOAEL would be a more scientifically sound basis for calculating the RfD. The current scientific thinking, supported by guidance documents, suggests that these skeletal anomalies are not adverse findings especially when they are not associated with any other malformations. Interpretation of these variations should be done in the context of other findings in a weight of evidence approach. In this study, there were no other findings to suggest that these findings were adverse.

Lauren Zeise

As indicated in response to Question 1 (A) above, the kidney hemosiderin deposit would be an alternative critical endpoint.

The fetal skeletal anomalies in the Khera study included delayed ossification and missing or unossified sternbrae. The identification of these indicators of altered growth would not be inconsistent with the Agency's "Guidelines for Developmental Toxicity Risk Assessment."

The justification indicates uncertainty regarding the toxicological significance, and indicates wavy or extra ribs and delayed ossification as most commonly observed. On a fetal (as opposed to litter) basis the occurrence of missing or unossified sternbrae ($p < 0.01$ second highest dose, $p < 0.0001$ high dose) and delayed ossification of the calvarium ($p < 0.001$ high dose) are statistically significant. The EPA Guidelines do not preclude the analysis of such skeletal formations on an individual rather than litter basis (Guidelines for Developmental Toxicity Risk Assessment, p. 13).

(A) Oral Reference Dose (RfD) for Biphenyl

Question 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, 2000)? 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?

Scott M. Bartell

Benchmark dose modeling was appropriately conducted and clearly described. In fact, this is the most transparent and detailed description I've ever read from EPA regarding model fitting procedures using BMDS. However, the rationale for choosing a 10% BMR isn't entirely clear... Section 5.1.2 indicates that "a BMR of 10% extra risk among affected litters was employed in order to better approximate a 5% extra risk in affected offspring," but doesn't explain why a 10% extra risk among affected litters reasonably approximates a 5% extra risk in affected offspring. A clear explanation and/or citation would be helpful here.

John M. Cullen

The response to this question is related to the question and my response above. The main contributing data in the selection of litters (not individuals) with any skeletal anomalies is the incidence of delayed ossification and wavy or extra ribs. This may not be an appropriate choice for this determination and the renal lesions (other than hemosiderin deposition) may be a superior point for evaluation.

Brant A. Inman

I actually had never heard of BMD prior to my review of this document. This is likely because I am a clinician and not a toxicologist. I did review the source document (EPA 2000) on the EPA website (<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=22506#Download>) and was interested to note that the document that I downloaded from the EPA specifically states on its cover page that it is a "draft" and "do not cite or quote." This led me to wonder about the validity of the citation. Also, it was 96 pages long. To learn more about the method, I ultimately ended up reading the 10 page paper by Davis et al. [Toxicol Appl Pharmacol 2011], which was a much better source of information since it was far more concise. Consider referencing this paper.

The 10% extra risk (not added risk which is different) was explained, though why the value was set at 10% and not 5% or 7.5% (for example) was not explained. The use of BMR for calculating the POD was adequately explained.

Frederick J. Miller

The BMD modeling procedure was adequately described for the most part, and the appendices provided ample data to support the conclusions presented in the main body of the document. For

one of the criteria for determining an adequate fit (i.e., the criterion that a value of <2 was obtained for the largest scaled residual for any data point in the data set including the control) could be better explained. My assumption is that this is basically equivalent to showing that the model does not predict values outside of an approximate 95% confidence interval for individual observations. If that is the intent, the authors should better clarify what is meant by this criterion. Why a 10% extra risk level rather than the more typical 5% extra risk was used was adequately explained on p. 80 of the document. Because of the correlation of individual pups within a litter, the Agency used 10% extra risk to better approximate the litter as the experimental unit since the Khera et al. (1979) study reported affected pups within litters, which prevented the Agency from using nested models.

Ricardo Saban

Benchmark dose modeling was appropriately conducted and clearly described.

Mary Alice Smith

Because there was ‘frank maternal toxicity’ at the highest dose, was it included in the BMD calculation? It is not clear whether it was included or not. If so, it should be justified.

Paul W. Snyder

As indicated previously, the Khera et al. (1979) study should not be included in the BMD modeling for the reasons stated above.

Lauren Zeise

The modeling follows EPA guidance. Its use of a 10% rather than a 5% risk as a point of departure is reasonable and adequately justified in the text. However, the argument provided to support not applying cross species scaling to the oral dose is problematic. It is argued that there are difficulties in scaling when it is to be done across individuals at different life stages. That is not what is being done here. It is the dose to the dams that is the basis for the modeling, not the dose to the fetus. The dose to the adult rat dams in the developmental toxicity study would be scaled to a human dose using the default human bodyweight and the dam bodyweight. This is because the RfD only addresses the fetus via maternal exposure. The use of a factor of 10 for cross species adjustment instead of allometric scaling plus a pharmacodynamics factor is fine, but the discussion at the bottom of page 85 is problematic.

(A) Oral Reference Dose (RfD) for Biphenyl

Question 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.

Scott M. Bartell

The uncertainty factors are clearly described and consistent with the EPA (2002) recommendations.

John M. Cullen

The explanation of the selection of the uncertainty factors appears to be reasonable and based on the available (or lack thereof) data, given that this is not my area of expertise.

Brant A. Inman

On my first reading of the document I found the uncertainty factors quite confusing. Again, this may be due to my lack of knowledge pertaining to toxicology methodology. After reading Dourson et al. [Reg Toxicol Pharmacol 1996], I had a much better understanding of what the UFs were and what justified their use.

Frederick J. Miller

This reviewer agrees with the choice of the values for the various uncertainty factors except for the intraspecies uncertainty factor. The intraspecies uncertainty factor can be divided into a dosimetric and sensitivity components. This reviewer has shown that the UF for intrasubject dosimetry variability is at most 1.3 for particles and most probably holds for gaseous chemicals as well. This is even taking into account the role of functional residual capacity for absorption of gases and deposition of particles, as well as the mass median aerodynamic diameter and geometric standard deviation in the case of particulate aerosols, on the delivery of inhaled compounds to the lower respiratory tract. Hence, the intraspecies UF is more likely a value of 3.9 than it is a value of 10. That being said, this observation is a result of dosimetry comparisons for many inhaled particles and gases, but has not specifically been made for biphenyl. Thus, this reviewer cannot fault the Agency for using a value of 10, also given that there is not adequate pharmacokinetic data of the distribution and elimination of biphenyl from the body.

Ricardo Saban

Outside my expertise.

Mary Alice Smith

The selection of the uncertainty factors is appropriate.

Paul W. Snyder

Yes, no further comment.

Lauren Zeise

The factors of 10 for interspecies and intraspecies adjustments are appropriate. The justification of factors of 1 for LOAEL to NOAEL and database deficiencies require more discussion. The statement that skeletal anomalies are assumed to represent minimally biologically significant change requires further justification. The discussion of database deficiencies lists the many studies performed, but a number of these were limited by small numbers of animals, incomplete histopathology, and insufficient study length. The case is again made that the neurological effects observed epidemiologically have not been observed in animal studies (p. 87). However, the discussion does not describe how those studies could have picked up such effects and there is no developmental neurotoxicity study. Also, differences between oral and inhalation routes may be involved. A factor of 3 or 10 for database uncertainty could be justified.

(B) Inhalation Reference Concentration (RfC) for Biphenyl

Question 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.

Scott M. Bartell

The lack of suitable data for deriving an RfC is evident and well described in the toxicological review.

John M. Cullen

I agree that there is insufficient data to evaluate an inhalation RfC of biphenyl. I am not aware of any other data related to this issue.

Brant A. Inman

I am unaware of other available data that would allow the calculation of the RfC. I thought the text explained well that lack of data was the reason for no RfC calculation.

Frederick J. Miller

The arguments presented for not developing a RfC for biphenyl are clearly and reasonably presented. However, this reviewer does not agree with the last sentence on p. 89 that states “The lack of adequate data to derive an RfC represents a significant uncertainty for the evaluation of risks from exposure to inhaled biphenyl.” Here is where some information on ambient exposure levels could be used to put the likely risk into perspective by comparing ambient exposures to occupational exposure limits or exposure limits established by other organizations or countries. For example, the threshold limit value (TLV) for biphenyl is 1 mg/m³, which is a value that workers can be exposed to for 8 hours per day for a lifetime of work. The highest ambient concentration of biphenyl measured in Canada was 0.022 µg/m³ or 0.000022 mg/m³. This is a value 45 million-fold less than the TLV and implies that ambient exposure levels should not have any attributable risk for biphenyl exposures via the route of inhalation. Even though the Agency does not calculate an RfC, the Agency could well provide narrative that provides a perspective on the situation by comparing real world ambient air levels to TLV exposure limits for this compound.

Ricardo Saban

The lack of suitable data for deriving an RfC is evident and well described in the toxicological review.

Mary Alice Smith

The justification was well described.

Paul W. Snyder

No appropriate inhalation data were available or identified for biphenyl that could be used to derive an RfC.

Lauren Zeise

The decision not to derive an RfC has been adequately justified. There are insufficient data from inhalation studies. It is not recommended that extrapolation from the oral value be attempted because the pharmacokinetics associated with toxicity may be relatively complicated and there are no data on route differences in pharmacokinetics.

(C) Carcinogenicity of Biphenyl

Question 1. Under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005; www.epa.gov/iris/backgrd.html), 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.

Scott M. Bartell

This rationale for this characterization should be more clearly described. The following text in Section 4.7.1. is a good start: "The findings from these earlier studies [a list including Imai et al., 1983] were less informative for the carcinogenicity of biphenyl than Umeda et al. (2005, 2002) because of various study limitations. With the exception of Imai et al. (1983), these limitations include small group sizes and shorter-than-lifetime exposure durations due to design or decreased survival unrelated to tumor development." However, the draft review does not indicate what study limitations of Imai et al. (1983) make it less informative than the Umeda et al. studies.

Section 4.7.1. also includes the following unsupported statement: "The available evidence suggests that humans would be less susceptible to these [urinary bladder] tumors than rats (see discussion in Section 4.7.3.1.4.2)." I don't see any evidence comparing susceptibility between rats and humans. The most relevant evidence presented in Section 4.7.3.1.4.2 is that 1.) sulphate conjugation of hydroxylated biphenyl metabolites occurs in humans and 2.) urinary bladder calculi and bladder carcinomas are associated in white humans. Don't these facts suggest that humans could be susceptible to biphenyl-induced urinary bladder tumors? The discussion of potential roles of urinary pH and calculi residence time is interesting, but largely hypothetical.

Also in this section, the draft review leaps from the statement "when one takes into consideration information on the mode of action for biphenyl-induced tumors, risk of female liver tumors only is operative at environmentally relevant exposures" to the next sentence: "Accordingly, this assessment concludes that there is 'suggestive evidence of carcinogenic potential.'" Is the rationale for selecting the "suggestive evidence" category that the exclusion of urinary bladder tumors means that the relevant positive findings for biphenyl carcinogenicity apply for only one species, sex, strain, and site, thereby obviating the "likely to be carcinogenic" category? This should be explicit.

John M. Cullen

In my view, a summary overview of the data indicates that female BDF₁ mice had a treatment-related increase in hepatocellular adenomas and carcinomas, but not male mice in the study described by Umeda et al. (2005). These data come from the more recent and apparently best designed of studies of this type. In two additional studies, biphenyl did not produce hepatic neoplasia in either gender of ddY or other F1 generation mice of different types. Reticular cell sarcomas were identified during the group discussion to be significantly increased in one study of strain B female mice as well, although this was not identified in our report (NCI 1968). This is of limited significance because this pathologic term is no longer used and the nature of the

proposed neoplasia is not clear. Bladder neoplasia in rats following high doses of biphenyl, similar to the process observed with saccharin, is reasonably attributed to the formation of calculi, not a direct effect of biphenyl. Limited chronic studies in other species (dogs and primates) did not produce neoplasia or lesions that might suggest incipient tumor development, although these studies were of relatively short duration for assessment of carcinogenicity. Therefore, there is evidence that one sex and one species is affected and not all strains of mice are affected. There are no chronic inhalation studies available, consequently there is no evidence of neoplasia production by this route. Given these data, I do not agree with the designation of “suggestive evidence of carcinogenic potential” by all routes of exposure and or the exaggerated statement about this classification by adding “at environmentally relevant exposure levels in humans” on page 60. Using the terminology from the NTP carcinogenicity studies, I would favor a designation of “some evidence” of carcinogenicity based on the liver tumors in the BDF₁ female mice. I would support an effort to harmonize the terminology used by different governmental and international agencies given the diversity of options currently in use.

Brant A. Inman

Oral biphenyl causes liver and bladder tumors in rats and mice; the data clearly supports this. I am less clear about inhalational and dermatological routes of exposure since I do not see any data in the document to support that all routes of exposure are toxic.

Frederick J. Miller

According to the narrative in the 2005 EPA Cancer Guidelines, the descriptor of “suggestive evidence of carcinogenic potential” is warranted. However, the Agency has exaggerated the statement about this classification by adding “at environmentally relevant exposure levels in humans” on page 60 and again on page 99 when stating that the database for biphenyl provides “suggestive evidence of carcinogenic potential”. The effect level in female mice was 414 mg/kg-day. Even if one went to the lower level of 134 mg/kg-day, this exposure is about 79,000-fold greater (i.e., about 5 orders of magnitude) than that for the maximally exposed age category for humans (i.e., the Canadian data have 1.7×10^{-3} mg/kg-day for children ages 6 months to 5 years or 134/0.0017). To use the phrase “at environmentally relevant exposure levels in humans” is highly misleading and is factually incorrect. Moreover, if the Agency does not like the use of Canadian data, they should be able to find comparable kind of information for the U.S.

Ricardo Saban

There is evidence that female mice, but not male mice, present a treatment-related increase in hepatocellular adenomas and carcinomas. However, scanty is the literature supporting suggestive evidence of carcinogenic potential by all routes of exposure.

Mary Alice Smith

Although discussion of the studies was extensive, there was not enough synthesis of the data nor attention paid to possible confounders such as palability, weight loss, etc. The discussion of mode of action was helpful. Some discussion of what constitutes ‘environmentally relevant

exposures' is needed. As to the selection of carcinogenic endpoint and relevance to humans, I leave that to carcinogenic experts on the panel.

Paul W. Snyder

The body of scientific data across multiple species largely supports the conclusion that biphenyl is not carcinogenic. However, the increased incidence of liver tumors in female BDF₁ mice (Umeda 2005) would by default justify a conclusion of “suggestive evidence of carcinogenic potential” category according to 2005 EPA Cancer Guidelines.

Lauren Zeise

The carcinogenicity conclusion rests on the following findings: 1) liver tumors in the female mouse in the Umeda et al. (2005) study provide good evidence of carcinogenicity; 2) biphenyl absorbed by any route can distribute to target tissues; 3) other assays do provide conflicting results; 4) the bladder tumors seen in the rats in the Umeda et al. (2002) study do not have relevance to “environmentally relevant” doses.

Umeda et al. (2005) liver tumors in female mice. The finding of adenoma and carcinoma combined in the top two dose groups are over 5-fold greater than the concurrent control, and both are statistically significant. The finding in the lowest dose group is elevated and although not statistically significant ($0.05 < p < 0.1$) is consistent with what one would expect with increasing trend. All tumor findings in treated groups are above the largest incidence in historical controls. There is also a clear dose response, with a highly significant trend test ($p < 0.0001$). Thus, the finding of liver tumors in the female mouse in this study is robust.

The US EPA Guidelines provide examples of evidence for the descriptor “suggestive evidence”. The closest one to the case at hand is:

- “a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend.” (p. 2-56)

Clearly the robust finding in female mouse is stronger than this. There are significant findings in multiple groups and a strong overall trend ($p < 0.0001$). There is also the small piece of evidence coming from the Innes et al. study of reticular cell sarcoma in female strain B mice ($p < 0.030$), although given the limitations of this study it provides little additional weight.

It is also clear from human and animal observations that the liver is a target organ for biphenyl toxicity.

The finding of liver tumors in the female mouse in the Umeda et al. 2005 study in and of itself provides a sufficient basis for making a suggestive evidence conclusion, in the absence of compelling evidence that the findings are not relevant to humans.

Routes of exposure. The Review lays this out well. It might also be added to the items noted that the Sun 1977 study in the mouse also provided evidence of distal impacts with inhalation exposure – liver and kidney.

Differing results. The Toxicological Review does a good job laying out the basis for the liver tumor finding, why other studies have little weight (page 60). However, the monkey and dog studies should not have been included in this discussion (Dow 1953, Monsanto 1946) because they were not sufficiently long in duration to be considered informative for carcinogenicity determination. Also, the Innes NCI study does have positive finding for one sex strain so should not be treated as a completely null study. The discussion could go a little farther to lay out the deficiencies with the other studies – e.g., Pecchiai and Saffiotti fell short by one year of a standard cancer bioassay and only used 8 animals per group, Ambrose had only 15 animals per group, Shiraiwa was less than 1.5 years in length. The Review is correct to point out that Imai found no evidence of carcinogenicity and a reasonably good study in a different mouse strain than used in the Umeda study.

The EPA pointed out that the Imai study used a different strain. Thus, it does not represent “conflicting evidence,” because “[d]iffering results, that is, positive results in some studies and negative results in one or more different experimental systems, do not constitute conflicting evidence, as the term is used here.” (EPA Carcinogen Guidelines, p. 2-57). There were also other differences between the studies, but the upshot is that the overall evidence does not constitute “inadequate evidence.”

Bladder tumors. The treatment of bladder tumor findings as not contributing to the positive evidence at “environmentally relevant doses” was well described. An alternative approach would be to address the issue of high dose carcinogenicity via calculi formation in the dose response assessment. This would lead to an increase in the overall evidence call for carcinogenicity for high dose exposures. In several places in the document, the point is made that certain metabolites are minor (e.g., 2-hydroxybiphenyl) and therefore cannot be playing a role. This is not adequate justification for determining that a metabolite cannot contribute. Consideration of the potency and the extent of formation is required. EPA has done an elegant work in this area for other environmental agents.

(C) Carcinogenicity of Biphenyl

Question 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.

Scott M. Bartell

This is clearly described and I believe that it is a reasonable interpretation of the available data.

John M. Cullen

The presentation of the available data is clear and persuasive. The formation of calculi is well documented. Gender differences in urinary metabolites and electrolytes are identified to elucidate the mechanism of calculus formation. Studies demonstrating increased transitional cell mitoses support the proposed mechanism of tumor formation. Given previous studies with saccharin, which also precipitates in the urinary bladder, the proposed EPA conclusions are well reasoned and well supported.

Brant A. Inman

As a bladder cancer expert, this was the most critical finding in the toxicology review for me to assess. Several issues came to mind:

- The term “transitional cell carcinoma” was used repeatedly. This is an old terminology that has not been routinely used since 1998 when the WHO/ISUP meeting standardized terminology [Epstein et al. *Am J Surg Pathol* 1998]. Since then the term “urothelial carcinoma” has become the preferred terminology. See also *Eble J.N., Sauter G., Epstein J.I., Sesterhenn I.A. (Eds.): World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. IARC Press: Lyon 2004* for details.
- The data clearly show that there is an association between the development of bladder stones and bladder cancer with biphenyl exposure. However, chronic bladder stones (and other irritants) are typically associated with squamous cell carcinomas of the bladder rather than urothelial carcinomas. Why the discrepancy?
- It is certainly possible to have an exposure that causes both bladder stones and cancer. If this was true, then the model would be:

Biphenyl → Stones + Cancer

Rather than:

Biphenyl → Stones → Cancer

For this reason, the data simply do not prove that stones are required for carcinogenesis.

- I am unaware of another proven mode of bladder carcinogenesis for biphenyl.
- A question of whether the observed bladder effects are valid in humans is a secondary point. Clearly, some exposures that cause tumors in rodents don't do so in humans, at least not at the doses seen in human exposures. However, I do believe that the potential exists for there to be carcinogenicity and this should not be minimized.

Frederick J. Miller

The Agency adequately described and documented the reasons and the science behind why they judged that biphenyl-induced urinary bladder tumors in male rats are a high dose phenomenon involving sustained occurrence of calculi in the urinary bladder and a subsequent cascade of events.

Ricardo Saban

- 1- The report indicates that the best-supported hypothesis proposes a mode of action whereby the formation of urinary **bladder calculi** is the key event in the development of bladder tumors. However, there is not a clear explanation why the association between calculus formation and tumors is both gender and species specific.
- 2- It is not clear whether other mechanisms of action have not been explored.
- 3- Alternative mode of action. In a bladder cancer cell line (TSGH-8301), it was shown that 2-aminobiphenyl (2-ABP) up-regulated the expression of COX-2 in a dose- and time-dependent manner and that this induction is mediated through NADPH oxidase-derived ROS-dependent JNK/ERK-AP-1 pathways (Chen CC, Cheng YY, Chen SC, Tuan YF, Chen YJ, Chen CY, and Chen LC. Cyclooxygenase-2 Expression Is Up-regulated by 2-Aminobiphenyl in a ROS and MAPK-Dependent Signaling Pathway in a Bladder Cancer Cell Line. *Chem Res Toxicol* 25: 695-705, 2012).
- 4- NOT CLEAR. Several publications described in the report indicate that biphenyl **did not increase the incidence** of cancer promoted by known carcinogens (BBN or EHEN), whereas in rats it seems to potentiate. Nevertheless, biphenyl was considered by some of the authors a urinary bladder tumor promoter.
- 5- **The report did not provide a clear conclusion** on whether biphenyl represents a carcinogenic potential to humans. In some instances, the report indicates that "there is suggestive evidence of a carcinogenic potential" whereas in other instances it is indicated that "**biphenyl should not poses a risk of urinary bladder tumors at environmentally relevant exposure levels in humans.**"
- 6- The available information is insufficient to establish the mode of action **for noncancer health effects** following exposure to biphenyl. Damage to urinary tract seems to be due to precipitation of crystals. In this context. The induction of urinary bladder tumors in

F344 male rats by dietary biphenyl exposure is closely related to the formation of urinary bladder calculi.

The available data on the biphenyl mode of action are insufficient to conclude that biphenyl should not pose a risk of urinary bladder tumors at environmentally relevant exposure levels in humans.

Mary Alice Smith

This section was well described and justified.

Paul W. Snyder

The mechanism for calculi associated urinary bladder tumor formation is well documented in the literature and clearly presented in this draft report.

Lauren Zeise

That calculi are a risk factor for bladder carcinogenesis in humans and rats is well supported and is discussed adequately in the Review (although some suggested changes are noted below). That biphenyl produces bladder calculi in rats at high doses is also well described. The possibility that biphenyl metabolites could have contributed to the overall carcinogenesis process through genotoxicity remains and has been acknowledged in the Review, but discounted because levels of formation of the potentially genotoxic metabolites are small. While the evidence adequately supports calculi formation in the rat bladder as the major determinant of bladder carcinogenesis seen, a small contribution biphenyl metabolites via genotoxicity to the process cannot be ruled out. That does not preclude a conclusion that the observed rat bladder tumors would not have occurred without calculi formation.

(C) Carcinogenicity of Biphenyl

Question 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).

Scott M. Bartell

The determination is clearly described, and I certainly can't identify the mode of action.

John M. Cullen

I agree with the determination that there is insufficient information to identify the mode(s) of carcinogenic action for biphenyl in mice. The descriptions of investigations into possible modes of action such as genotoxicity, enzyme induction and adduct formation are well described. The possibility of reactive oxygen species is raised and may be pursued in the future, but there is insufficient data at this point to evaluate this possible mode of action.

Brant A. Inman

I agree that the mode of carcinogenesis is not clear for liver tumors.

Frederick J. Miller

The Agency does a good job on pages 71 to 73 of presenting the data in relationship to a mode of action hypothesis and explaining why the available data do not support the identification of the mode(s) of carcinogenic action for biphenyl-induced liver tumors in mice. They also point out that there are no data available to support a number of the criterion that an adequate data set should meet for a PPAR α mode of action.

Ricardo Saban

There is insufficient information to determine the mode of action for biphenyl-induced liver tumors in mice. Participation of peroxisome proliferation (PPARs) seems to be the primary mode of action being studied. However, there is no evidence of dose-response concordance and there is not adequate data supporting the peroxisome proliferation or alternative hypothesis.

Mary Alice Smith

The only concern about liver tumors was the lack of response in male mice. However, this was discussed and justified.

Paul W. Snyder

There are no scientific reports documenting the mode(s) of action for the formation of liver tumors in female mice.

Lauren Zeise

EPA has provided adequate justification for this conclusion. Data are lacking for a number of elements involved in a peroxisome proliferation mechanism, as described well in the Review.

(C) Carcinogenicity of Biphenyl

Oral Slope Factor (OSF)

Question 4. A two-year cancer bioassay of biphenyl in BDF₁ 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.

Scott M. Bartell

The rationale for selection of the Umeda et al. (2005) study is clearly described.

John M. Cullen

The study by Umeda et al. (2005) appears to be the most thorough and best designed chronic study available. The explanation for its use is generally clear and appears well supported; however, it would be useful to have a more detailed explanation and evaluation of the strengths and weaknesses of the other, earlier studies in mice and other species to assist in the assessment of the entire set of relevant data. I am not aware of another study that would be useful.

Brant A. Inman

This appears reasonable to me.

Frederick J. Miller

The selection of the Umeda et al. (2005) study of a 2-year cancer bioassay of biphenyl in BDF₁ mice is scientifically supported and clearly described by the Agency in the document as the study recommended for deriving the oral slope factor.

Ricardo Saban

The rationale for selection of the Umeda et al. (2005) study is clearly described.

Mary Alice Smith

The study was scientifically supported and described. The only concern is lack of response in male mice, as previously mentioned.

Paul W. Snyder

The study with the lowest NOAEL should be selected for derivation of the OSF.

Lauren Zeise

The liver tumors observed in female mice in the Umeda et al. (2005) study was the most relevant data set for estimating an oral slope factor. The selection of the study is scientifically supported and clearly described.

(C) Carcinogenicity of Biphenyl

Oral Slope Factor (OSF)

Question 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.

Scott M. Bartell

The rationale for selection of liver tumors in female mice is clearly described.

John M. Cullen

Given the statistical increase in liver neoplasms in female mice, this appears to be the most useful data to use for the derivation of the OSF. Female BDF₁ mice were the only animals to develop neoplasia following oral exposure to biphenyl, excluding the male rats with urinary calculi-induced transitional cell neoplasia, making their results the only reasonable choice for the derivation of the OSF.

Brant A. Inman

I would have considered using the urologic toxicity data given that (i) liver tumors form more easily in mice, (ii) liver tumors occurring almost exclusively in females, (iii) urinary toxicity has been consistently observed in all studies at high levels, (iv) bladder tumors were common causes of animal death. On the other hand, the retrospective human studies argue that liver toxicity is the predominant high dose immediate toxicity.

Frederick J. Miller

Of the available data, the Agency focused on the combined incidence of adenoma and carcinoma liver tumors in female mice to derive the oral slope factor. While there is not a different cancer endpoint that one could recommend, the Agency has not adequately defended their choice in that there is no discussion in Section 5.4.3 on the major discrepancy in the selected study between the results seen in male mice versus female mice. The discussion on pages 35 and 36 of this discrepancy is not brought forward in the consideration of the reasonableness of calculating an oral slope factor. There is a clear decrease in tumor incidence with increasing dose in the male mice and just the opposite situation in female mice. So the Agency just picked the female data. From a teleological perspective, one would not expect biphenyl exposure to be good for a person; however, at a minimum the Agency should note this discrepancy and discuss the implications for the usefulness of the resulting oral slope factor.

Ricardo Saban

The rationale for selection of liver tumors in female mice is clearly described.

Mary Alice Smith

Why the adenoma or carcinoma data were combined for the calculations of the OSF is not well described. It would seem that the adenoma data would be appropriate, particularly because carcinoma was not statistically different from control at the high dose.

Paul W. Snyder

The study selected had a finding that was only present in one gender that was confounded by a low incidence in the concurrent controls. Consideration of another study is warranted, but this reviewer is not sure what study should be used in light of the limitations already discussed for other studies.

Lauren Zeise

The liver tumors observed in female mice in the Umeda et al. (2005) study was the most relevant data set for estimating an oral slope factor.

(C) Carcinogenicity of Biphenyl

Oral Slope Factor (OSF)

Question 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, 2000)? 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?

Scott M. Bartell

The benchmark dose modeling for liver tumors is clearly described and well supported. However, on page 94, line 22 (and perhaps line 17) I believe the text should read “the multistage-cancer model” rather than “the multistage model.” If I recall correctly, the two models use the same functional form, but BMDS forces the betas in the multistage-cancer model to be nonnegative, ensuring a monotonic dose-response curve. This may partly explain the poor fit when the high dose group is included. An unrestricted multistage model might work with the entire data set, but the multistage-cancer model without the highest dose group may be preferable on theoretical grounds if high-dose hormesis is implausible.

John M. Cullen

The explanation appears to be clear, but this area is well outside of my expertise and I cannot provide a critical assessment.

The choice appears to be clearly described, but, as above, this area is well outside of my expertise and I cannot provide a critical assessment.

Brant A. Inman

The liver BMD methods are clear and appear appropriate.

Frederick J. Miller

Pages 93 and 94 of the “Toxicology Review of Biphenyl” document clearly describes and supports why a linear low-dose extrapolation from the point of departure was used as well as why the use of 10% extra risk of incidence of liver tumors was used in assessing the benchmark response.

Ricardo Saban

The benchmark dose modeling for liver tumors is clearly described and well supported.

Mary Alice Smith

The selection of models and calculations of HED appears to be appropriate and was clearly described. The selection of 10% extra risk was justified.

Paul W. Snyder

No comments.

Lauren Zeise

The modeling has been appropriately conducted and is clearly described. The EPA followed appropriate procedures in addressing the lack of fit.

(C) Carcinogenicity of Biphenyl

Oral Slope Factor (OSF)

Question 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.

Scott M. Bartell

This nonlinear approach is reasonable and consistent with the evidence. However, because the RfD is derived from a developmental endpoint rather than calculi formation, I would add a (very brief) explicit comparison between the RfD and the NOAEL for calculi formation. I'm sure it's far below the NOAEL but it would be best to demonstrate that.

John M. Cullen

The explanation for the non-linear approach is clear and well-reasoned. Lesions of the bladder (and the kidney's transitional epithelium) are very likely attributed to the formation of calculi. Given that calculi only develop in significant numbers at dosage levels that are quite high and over a demonstrated threshold, the current approach is appropriate.

Brant A. Inman

The data suggest, but certainly do not prove, that a multistep carcinogenic process is occurring. This was noted in my comment above. While stones may be a contributing factor to bladder carcinogenesis, I do not think that they are likely to be sufficient to cause bladder cancer on their own. In other words, I suspect that (i) chronic irritation to the urothelium from stones plus (ii) continued urothelial exposure to toxic chemicals is resulting in cancer.

Frederick J. Miller

The decision by the Agency to use a nonlinear approach for extrapolating cancer risk from male rats to humans is clearly a defensible and scientifically supported decision. Section 4.7.3.1 (Mode-of-Action Information for Bladder Tumors in Male Rats) does an excellent job of discussing the relevant issues and presenting the data that led to the Agency's decision.

Ricardo Saban

The approach is scientifically sounded and clearly described.

Mary Alice Smith

This approach was supported with the scientific results and was well described.

Paul W. Snyder

The approach is scientifically supported and clearly presented.

Lauren Zeise

There has already been a judgment that these tumors would not occur at environmentally relevant doses. Given the nature of the hazard identification decision, modeling of this endpoint is not justified nor needed.

(C) Carcinogenicity of Biphenyl

Inhalation Unit Risk (IUR)

Question 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.

Scott M. Bartell

I am not aware of any such data.

John M. Cullen

I am not aware of any studies other than those cited in the report that describe experimental inhalation of biphenyl.

Brant A. Inman

The paucity of data supports the lack of IUR.

Frederick J. Miller

This reviewer does not know of any available data to support the derivation of an inhalation unit risk and did not find any such data via a Google search.

Ricardo Saban

I am not aware of any such data.

Mary Alice Smith

I am not aware of other data.

Paul W. Snyder

No adequate inhalation studies provided or found to support the derivation of an IUR.

Lauren Zeise

The decision not to derive an IUR is justified. An alternative would be to derive a IUR from the oral slope factor. Because little is known about route differences in pharmacokinetics and because there is no information on inhalation pharmacokinetics, the estimate would be uncertain.

V. SPECIFIC OBSERVATIONS*Scott M. Bartell*

Page	Paragraph or Line #	Comment or Question
18 and throughout		The meaning of the phrases “most quantitative” and “less quantitative” is unclear. Perhaps the writers meant “most precise” and “less precise?”
22		“...male rates have relatively higher urinary potassium concentrations and pH values than female rats.” Do humans exhibit the same differences? At least one study reports higher pH among human males than females (Pigoli et al., 2010), but it’s unclear to me whether the differences are large enough to be relevant to calculi formation.
		Table 4-4, combined transitional cell lesions for males at 4,500 ppm is shown as not statistically significant compared to the control group, which is implausible for 45/50 responders vs. 0/50 responders. The fisher.test function in R indicates that 2-tailed $p < 0.001$.
		Table 4-8, necropsy liver nodules among females at 6,000 ppm is shown as not statistically significant compared to the control group, which is implausible for 26/49 responders vs. 7/50 responders. The fisher.test function in R indicates that 2-tailed $p < 0.001$.
		Table 4-8, basophilic cell foci among females at 6,000 ppm is shown as statistically significant ($p < 0.05$) compared to the control group, but the fisher.test function in R indicates 2-tailed $p = 0.059$. Note: some statistical packages use an older method of two times the 1-tailed p-value, which results in 2-tailed $p = 0.11$. Neither method produces 2-tailed $p < 0.05$.
		Table 5-1, mineralization among males at 4,500 ppm is shown as statistically significant ($p < 0.05$) compared to the control group, but the fisher.test function in R indicates 2-tailed $p = 0.07$ (using either method).
		Table 5-1 and throughout, why is Fisher’s exact test used for some 2x2 tables and Pearson’s chi-squared test for others? The usual guidance is to use Fisher’s exact test when some expected counts are less than 5, or conservatively to use Fisher’s exact test for <i>all</i> 2x2 tables. It is strange to use Fisher’s exact test <i>only</i> for mineralization among males in Table 5-1, relying primarily on chi-squared tests for the other comparisons in that table, while relying only on Fisher’s exact test in other tables.
		Table 5-10 is wonderful—very concise and useful.

John M. Cullen

Page	Paragraph or Line #	Comment or Question
38	8	Clarify “entire body wall.”
42	36	H&E stained-spell out first time.
44		How was degeneration of Kupffer cells identified or classified?
46	24	Kg should be mg.
	35	Define AP and other enzymes measured.

Brant A. Inman

Page	Paragraph or Line #	Comment or Question
9	3.3.1.1	The hypothesis that gender-specific urine pH is responsible for the higher urinary stone rate could be tested by feeding the rats different diets that affect urine pH.
	Throughout	Change “transitional cell carcinoma” to “urothelial carcinoma”

Frederick J. Miller

Page	Paragraph or Line #	Comment or Question
	All Tables	It would be helpful to the reader if the study from which the data arose was either included in the table title or in a footnote to the table. This would help the reader better identify with the multiple discussions that follow using the data in the various tables.
61	6 & 10	There is neither a Section 4.7.3.1.4.2 nor a Section 4.7.3.2.2.1.
68		The summary of bladder tumors in rats is redundant material. The authors have already stated the main points of the summary.
70	7	Section 4.7.3.2 – There might be some arguments laid out in a Critical Reviews in Toxicology article (38:857-875, 2008) that could apply to biphenyl that would be worth including here.
81	6	The Agency drops the highest dose and repeats the entire modeling procedure if their various procedures fail to produce a model that has an adequate fit to the data. Has the Agency ever examined the potential strategy outlined in Shirley (Biometrics 33:386-389, 1977) to achieve a monotonic increasing ordering of the data and then applied their modeling approaches? It would be interesting to compare the BMDL results using such an approach with the value obtained after dropping the highest dose.
95	Table 5-9	The footnote says Appendix C, but it should be Appendix D.

Page	Paragraph or Line #	Comment or Question
D-2	Table D-2	The Agency needs to be consistent when presenting the value of the cancer slope factor. Table D-2 gives the cancer slope factor as 0.008 while Table 5-9 gives it as 8.2×10^{-3} and on page 100 it is listed as 8×10^{-3} .
C-3	Table C-4	The Multistage (3-degree) model was selected because the AIC was the lowest with a value of 92.76. The Probit model had an AIC of 92.76 (i.e., a value only 0.26 % higher). Yet the BMDL10 for the multistage is 126.95 vs. 173.76 for the Probit, which is a 37 % difference. While I seriously doubt that the two AIC values are statistically different, the resulting BMDL values are likely to be so. The Agency should discuss the implications of these types of findings instead of a rote application of using the model that has the absolute minimum AIC.
	Appendix C	The Wald confidence intervals on parameter estimates are presented for the Logistic, Gamma, Power, and Polynomial (2nd) models but not for any other models. What is the reason for this? Is it that the other models do not support a standardized normal distribution or what? It would be helpful if the Agency clarified the output in this area.

Ricardo Saban

Page	Paragraph or Line #	Comment or Question
81	27-29	Unnecessary repetition. Compare with page 80 lines 10-12.
81	38	This is a repeat of page 81, lines 5-6.
82	1-3	This is a repeat of page 81, lines 5-6.

Mary Alice Smith

Page	Paragraph or Line #	Comment or Question
4	Last line	Annual US production is given from 1990; more recent data would be better.
6	20-21	Wording is confusing, did they use different vehicles?
15	3 rd from bottom	Two ‘((‘
31	Footnote to table	Is a Student's t-test appropriate for this analysis or should they compare all groups to each other? Or perhaps use a Dunnett's?
32	23-24	Should a study be used when survival was poor in the control animals?
39	33-34	This study is questionable for use because of crystallized test material during first few days of exposure and variability.
59	38	Was nervous system examined?
60	10-12	How does this relate to humans?
64	3-6	Is this relevant to humans?

Page	Paragraph or Line #	Comment or Question
66	16	Could not find data in Tables.
89	20-21	Need citation concerning toxicological significance of skeletal anomalies.

Paul W. Snyder

Page	Paragraph or Line #	Comment or Question
33	24, 25	Body weights should be mg not kg.

Lauren Zeise

Page	Paragraph or Line #	Comment or Question
10	Last full line	Indicates pattern in DBA/2Tex mice was unaffected, but should also include a remark regarding magnitude.
12	Figure 3-1	A possible improvement would be to adapt the figure to show the redox cycling discussed at the top of page 13.
13	Header 3.3.3.1	The header name could be misleading because the reader might take this to mean the extent that biphenyl induces phase I and II enzymes. The section is more about the identification of CYPs involved in biphenyl metabolism through studies using various inducers, and also the potential for coordinated induction by external agents to catalyze phase I and phase II metabolic processes.
20	5	A paragraph break is needed at the start of the study on Parkinson's disease (PD).
20	Discussion PD study	The calendar years of exposure for the Wasterman study and some comment regarding why exposures would be expected to be similar between the Hakkinen study (reported in 1973) would be helpful.
21-22	Introduction to 4.2	The paragraph introducing the material at the beginning of section could be more concise and better organized. It goes too much into the details of the carcinogenicity bioassay information (e.g., dose levels, tumor results) and interpretation of it, and does not introduce the material in the same order as in the subsections that follow.
22	14	Year of the Dow Chemical study should be given in the reference parenthetical.
22	19-22	Sentence on dose calculations would work better in the previous paragraph, or as a standalone paragraph, leaving the paragraph it is now in to discuss the results.
22	19-22	Bodyweight and food consumption values should be provided.
23	1-3	Sentence on dose calculations would work better in the previous paragraph (as was done with the Shiraiwa study), or as a standalone paragraph, leaving the paragraph it is now in to discuss the results.
23	1-3	Bodyweight and food consumption values should be provided.

Page	Paragraph or Line #	Comment or Question
23	29-35	Same comment as above regarding separating dose calculation from results with a paragraph break.
27	7-8	Terms NOAEL and LOAEL introduced earlier and were not defined. Here they are defined.
27	10	A paragraph break is needed. The beginning of discussion of the Shiraiwa study should not be included in the summary of the Umeda study.
30	9	Suggest inserting a paragraph break between the dose calculation piece and the reporting of study results.
28	30	The study by design was 75 weeks, or 1.4 years in length, less than standard 2 year study. This may have precluded the observation of late occurring tumors and should be noted.
32	22	Same comment as above regarding separating the dose calculation from results reporting.
33	5	Justification of severity score 3 versus 2 as adverse versus non-adverse is needed, and an indication of whether it was the study authors or the EPA.
38	Table 4-9	The numbers of animals in the control and treated groups differ by a factor of roughly 4. It would be easier to see differences in incidence if the incidences in percent were also reported parenthetically.
37	19	The incidence of reticular cell sarcoma in female Strain B mice is significantly greater in the biphenyl treated animals than the controls by the Fisher Exact test. This should be noted in the text and in Table 4-9.
38	17	Further group size in the rhesus monkey and dog study is small – a single female per dose group and two males per dose group. This major study limitation deserves note as the studies are being presented. These studies are conducted for less than 1/10 the animals' lifespan should not be characterized as chronic studies and would be better placed in the subchronic section.
39	2	
39	5	Introductory text could indicate the nature of the inhalation studies to follow, pointing out that what is to be discussed is a series of subchronic rodent studies, and that there are no chronic inhalation studies available.
48	2	Would be good to report the dose in mg/kg.
50	17	It would appear that the LOAEL and NOAEL for the study would be 248 and approximately 60 mg/kg based on stones in the urinary tract. The paragraph on findings did not include a reporting of incidence of stones in the low dose group; this should be added.
55	Table 4-14	There is a statistically significant response in the Innes/NCI study in female strain B mice for reticular cell sarcoma ($p < 0.03$, Fisher Exact). The observed incidence in the treated is four-fold that in the control.
		The first column dog study should indicate 2 males per group.

Page	Paragraph or Line #	Comment or Question
		<p>The mg/kg-d dose for the dog study should be given.</p> <p>As noted above, because the one year dog and rhesus monkey studies are for a short period of the animals' lifespan, they would be more appropriately placed in a different section of the table and text. They are not really chronic studies.</p>
59	36-38	The statements regarding neurotoxicity do not address mode of action. The animal studies were not designed to detect the type of neurotoxicity observed in humans.
61	5	The relationship between the occurrence of bladder stones and bladder cancer is recognized on page 69. Here on page 61 is important to be clearer about the reason why EPA considers the bladder tumor findings to be not relevant to low human exposures of biphenyl.
61	38	The Sun 1977 study in the mouse also provided evidence of distal impacts with inhalation exposure – liver and kidney.
B-8	Table B-2	The route of administration should be included for the in vivo studies in the table. Also Sasaki et al. (1997) involved serial sacrifice at different time points.
63	25	Smith et al. used OPP not biphenyl.
68	33	The statement that the preponderance of evidence supports a mode of action only involving calculi is too strong. The statement that the preponderance of evidence supports calculi as the predominant mode of action would be correct.
69	9	The sentence regarding bipedal humans and clearing of bladder calculi appears to discount bladder calculi. Further on the point is made that they are a risk factor for transitional cell carcinomas. They are also recognized as a risk factor for squamous cell and small cell carcinomas and this can be added.
95	8	While a PBPK model is useful for route to route extrapolation, it is not essential. But it is lack of information on pharmacokinetics that makes route extrapolation for this case particularly uncertain.
Appendix B and elsewhere		The 1997 in vivo oral study of Sasaki et al. is of interest because the largest findings for DNA damage – in all seven tissues examined - occurred at the 24 hour time point, and not earlier 3 and 8 hour time points. Those authors attributed it to biphenyl metabolism to phenylbenzoquinone. Some further discussion of the Sasaki et al. study could be included that lays out the interim sacrifice findings. This could be included in some detail in Appendix B and referred to in the genotoxicity section. The genotoxicity of this compound and metabolites has not been thoroughly explored and some note of this is needed.